(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 092 764 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
18.04.2001 Bulletin 2001/16

(51) Int. Cl.[7]: **C12M 1/36**

(21) Application number: **00121663.9**

(22) Date of filing: **04.10.2000**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **11.10.1999 EP 99120289**
08.09.2000 EP 00119676

(71) Applicant:
F. HOFFMANN-LA ROCHE AG
4070 Basel (CH)

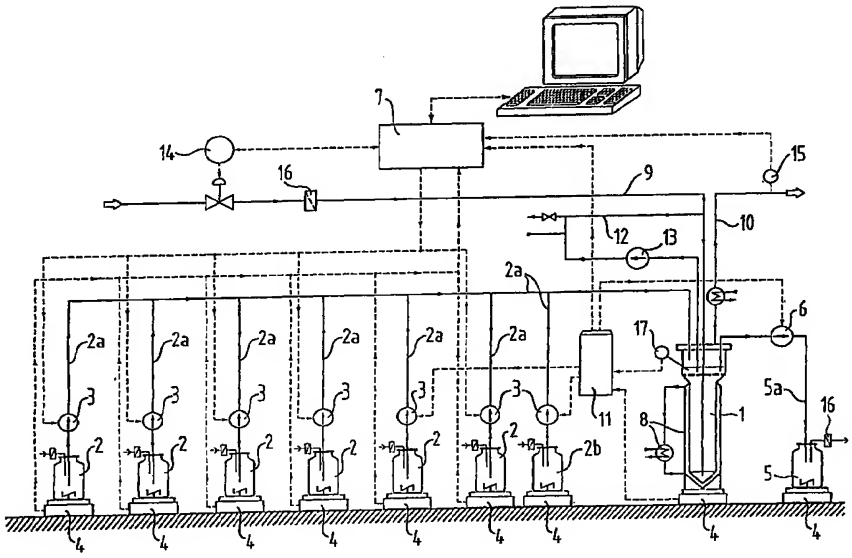
(72) Inventors:
- **Bartok, Attila**
8002 Zürich (CH)
- **Müh, Thorsten**
51375 Leverkusen (DE)
- **Rückel, Markus**
82377 Penzberg (DE)

(74) Representative:
Müller, Ingrid, Dr. et al
Grenzacherstrasse 124
CH-4070 Basel (CH)

Remarks:
The sequence listing, which is published as annex to the application documents, was filed after the date of filing. The applicant has declared that it does not include matter which goes beyond the content of the application as filed.

(54) Continuous fermentation process

(57) The invention is concerned with a continuous process for the manufacture of proteins by means of protein-producing microorganism in which process the microorganism is optionally immobilized on a solid carrier and/or the nutrients and other agents required for the growth of the microorganism and the optimal production of protein are fed into the reactor individually at a constant dilution rate. Furthermore, the invention is concerned with a process for the manufacture of proteins using a fermentation assembly.



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Description

[0001] The present invention relates to a continuous process for the manufacture of proteins.

[0002] In accordance with the present invention it has been found that splitting of cultivation media used in a continuous fermentation process allows to study the influence on growth and metabolite-production of microorganisms and thus to determine optimal conditions for the fermentation process. A continuously delivered fermentation medium can generally be split into as many fractions as it contains ingredients. Examples of such ingredients are carbon, nitrogen, phosphorus and sulfur sources as well as vitamins and complex substrates such as corn steep, yeast extract and other natural products. Furthermore, every required mineral, micro- or trace element can be provided separately as a solution of a water-soluble salt, such as a chloride, sulfate or nitrate. In this manner a fermentation medium of any desired composition can be obtained, provided that the desired amounts of the ingredients are (water)-soluble and no disturbing interactions (e.g., precipitation, reaction) occur in the individual feed solutions or in the fermentation medium.

[0003] In one aspect, the present invention is concerned with a continuous process for the manufacture of proteins by means of protein-producing microorganism.

[0004] More particularly, the invention is concerned with a continuous process for the manufacture of proteins by means of protein-producing microorganism in which process the microorganism is optionally immobilized on a solid carrier and/or the nutrients and other agents required for the growth of the microorganism and the optimal production of protein are fed into the reactor individually at a constant dilution rate.

[0005] In a preferred aspect, the invention is concerned with a process for the manufacture of proteins using a fermentation assembly that comprises

a vessel suitable for carrying out reactions involving living or inactivated cells;

at least two storage flasks connected to said vessel for supply of liquids and means to transport said liquids from said storage flasks to said vessel;

individual appliances monitoring the supply of the contents of said storage flasks to said vessel;

a harvest flask connected to said vessel and means to transport fermentation broth from said vessel to said harvest flask; and

a device for controlling and maintaining a constant dilution rate in said vessel with varying rates of individual supply of liquid from said storage flasks to said vessel.

[0006] Any conventional fermentation vessel can be used for the purpose of this invention. The vessel may be made of materials such as stainless steel, glass or ceramics and may have a volume of from e.g., l00 ml to 2500 m^3 although these figures are not critical to the invention. For continuous operation the inside of the vessel is optionally equipped with, e.g., a receptacle or sieve plate for uptake of immobilized cells. Further, the fermentation vessel is connected to a series of storage flasks that contain nutrient solutions and solutions for maintaining and controlling a desired pH and other parameters, such as foam formation, redox potential etc. in the fermentation broth. Depending on the particular needs of the fermentation, there may be separate storage flasks for individual supply of substrates that serve as carbon or nitrogen or mineral source for the living cells.

[0007] It has been found in accordance with the invention that the process is advantageously carried out at a constant dilution rate in the fermentation vessel. As used herein, the term "dilution rate" denotes the total volume of liquids supplied to the fermentation vessel per volume of the fermentation vessel per hour [h^{-1}].

[0008] Accordingly, it is a particular feature of the present invention to carry out the fermentation process at a constant dilution rate in the fermentation vessel while varying the supply of individual nutrient components or other additives during the fermentation process. To facilitate this task a storage flask containing an inert component, e.g., water is optionally provided that allows to complement the supply of liquids thus keeping the total supply of liquid constant.

[0009] The assembly that is preferably used to carry out the process of this invention further comprises means to transport the individual components of the fermentation medium from the storage flasks to the fermentation vessel, and appliances for monitoring the amount of liquid supplied to the fermentation vessel. Every combination of measuring instruments (e.g., volumetric or mass flow rate by either gravimetric, anemometric, magnetic, ultrasonic, Venturi, J, cross-relation, thermal, Coriolis, radiometric) and transfer units (e.g., pumps or pressure difference) can be used for this purpose. Additionally, every transfer unit can be applied as a dosing unit (e.g., gear, peristaltic, piston, membrane or excenter pump). For operation on small scale the supply is suitably monitored by weighing the storage flasks that contain nutrient or additive solutions in a predetermined concentration.

[0010] The device for controlling and maintaining a constant dilution rate in the fermentation vessel is suitably a sys-

tem comprising a measuring instrument that monitors the flow from the storage flasks and a controlling unit, e.g., a computer-software control that calculates the actual mass flow rates, compares them to the desired value and adjusts the pump setting accordingly. An appropriate system is, e.g., the Process Automation System, National Instruments, Bridge View, USA, for Windows NT 4.0 (represented by National Instruments, Sonnenbergstrasse 53, 5408 Ennetbaden, Switzerland) that is connected to the various operating units (scales, pumps) through a serial-interface box (Rocket Port, Comtrol Europe Ltd, Great Britain, represented by Technosoftware AG Rothackerstrasse 13, 5702 Niederlenz, Switzerland).

[0011] An assembly that can be used in the process of this invention is depicted in Figure 1.

[0012] The fermentation vessel 1 (Fermentor) is equipped with inlet tubes 2a from storage flasks 2 (suitably equipped with a stirrer) for supply of salt solution (Salts), nutrient solution (Nutrients), particular substrates (Substrate 1 and Substrate 2) for supply of, e.g., distinct carbon sources, agent for controlling the pH (Base), water for controlling a constant dilution rate, and antifoam. Pumps 3 transport liquids from the storage flasks 2 to the fermentor 1. Scales 4 monitor the amount of liquids supplied to and discharged from the fermentor. Further, the fermentor has inlet tubes 9 for oxygen supply and outlet tubes 10 for exhaust controlled by untits 14 and 15. Pump 6 discharges fermentation broth via outlet tubes 5a to a harvest flask 5. A main controlling unit 7 monitors and steers the overall process. Controlling unit 11 monitors and steers individual control systems 17 for temperature, pH, gas pressure, fermentor content and supply of antifoam agents. Circuit 12 including pump 13 is used for taking samples from the fermentation broth and for providing a controlled gas flow for moving the fermentation broth. Inlet and outlet gas flow is controlled by flow control 14 and 15. Sterile filters 16 are provided optionally. Optionally, the fermentation vessel 1 is equipped with a thermostating unit 8.

[0013] In the process of the present invention, any protein-producing microorganism either natural, e.g. fungal origin or bacterial origin or microorganisms which have been transformed by protein encoding DNA whereby such transformed microorganisms can be bacteria or fungi or yeasts, preferably from the genus Peniophora, Aspergillus, Hansenula or Pichia, especially *Aspergillus niger*, *Aspergillus awanari*, *Aspergillus sojae*, *Aspergillus oryzae* or *Hansenula polymorpha* or *Pichia pastoris*.

[0014] In this context, the skilled person in the art selects such a protein-producing microorganism which is known to be useful for the production of a desired protein.

[0015] In a preferred embodiment of the present invention the protein is selected from the group consisting of proteins having the activity of an enzyme such as catalase, lactase, phenoloxidase, oxidase, oxidoreductase, glucanase cellulase, xylanase and other polysaccharide, peroxidase, lipase, hydrolase, esterase, cutinase, protease and other proteolytic enzymes, aminopeptidase, carboxypeptidase, phytase, lyase, pectinase and other pectinolytic enzymes, amylase, glucosidase, mannosidase, isomerase, invertase, transferase, ribonuclease, chitinase, and desoxyribonuclease. Furthermore, in a preferred embodiment of the present invention the protein is selected from the group of therapeutic proteins such as antibodies, vaccines, antigens, or of antibacterial and/or health-beneficial proteins such as lactoternin, lactoperoxidase or lysozyme.

[0016] It will be understood by those skilled in the art that the term "activity" includes not only native activities referring to naturally occuring enzymes or therapeutic functions, but also those activities or functions which have been modified by amino acid substitutions, deletions, additions, or other modifications which may be made to enhance or modify the desired activity, or the thermostability, pH tolerance and/or further properties.

[0017] In a most preferred embodiment of the invention the selected protein is a protein having the activity of a phytase.

[0018] Examples of proteins having the activity of a phytase are described in EP 684 313, EP 897 010, EP 897 985 or in Examples 6 to 16 and Figures 2 - 22 of the present invention.

Figure 2: Design of the consensus phytase sequence. The letters represent the amino acid residues in the one-letter code. The following sequences were used for the alignment: *phyA* from *Aspergillus terreus* 9A-1 (Mitchell et al, 1997; from amino acid (aa) 27), *phyA* from *A. terreus* cbs116.46; (van Loon et al., 1998; from aa 27), *phyA* from *Aspergillus niger* var. *awamori* (Piddington et al, 1993; from aa 27), *phyA* from *A. niger* T213; Mitchell et al. 1997 from aa 27), *phyA* from *A. niger* strain NRRL3135 (van Hartingsveldt et al, 1993; from aa 27), *phyA* from *Aspergillus fumigatus* ATCC 13073 (Pasamontes et al, 1997; from aa 25), *phyA* from *A. fumigatus* ATCC 32722 (EP 897 985; Figur 1; from aa 27), *phyA* from *A. fumigatus* ATCC 58128 (EP 897 985; Figur 1; from aa 27), *phyA* from *A. fumigatus* ATCC 26906 (EP 897 985; Figur 1; from aa 27), *phyA* from *A. fumigatus* ATCC 32239 (EP 897 985; Figur 1; from aa 30), *phyA* from *Emericella nidulans* (Pasamontes et al, 1997a; from aa 25), *phyA* from *Talaromyces thermophilus* (Pasamontes et al, 1997a; from aa 24), and *phyA* from *Myceliophthora thermophila* (Mitchell et al, 1997; from aa 19). The alignment was calculated using the program PILEUP. The location of the gaps was refined by hand. Capitalized amino acid residues in the alignment at a given position belong to the amino acid coalition that establish the consensus residue. In bold, beneath the calculated consensus sequence, the amino acid sequence of the finally constructed consensus phytase (Fcp) is shown. The gaps in the calculated consensus

sequence were filled by hand according to principals stated in Example 6.

Figure 3: DNA sequence of the consensus phytase-1 gene (*fcp*) and of the primers used for the gene construction. The calculated amino acid sequence (Figure 2) was converted into a DNA sequence using the program BACK-TRANSLATE (Devereux *et al.*, 1984) and the codon frequency table of highly expressed yeast genes (GCG program package, 9.0). The signal peptide of the phytase from *A. terreus* cbs.116.46 was fused to the *N*-terminus. The bold bases represent the sequences of the oligonucleotides used to generate the gene. The names of the respective oligonucleotides are alternately noted above or below the sequence. The underlined bases represent the start and stop codon of the gene. The bases written in italics show the two introduced *Eco* RI sites.

Figure 4: Alignment and consensus sequence of five *Basidiomycetes* phytases. The letters represent the amino acid residues in the one-letter code. The amino acid sequences of the phytases from *Paxillus involutus*, phyA1 (aa 21) and phyA2 (aa 21, WO 98/28409), *Trametes pubescens* (aa 24, WO 98/28409), *Agrocybe pediades* (aa 19, WO 98/28409), and *Peniophora lycii* (aa 21, WO 98/28409) starting with the amino acid residues mentioned in parentheses, were used for the alignment and the calculation of the corresponding consensus sequence called "Basidio" (Example 7). The alignment was performed by the program PILEPUP. The location of the gaps was refined by hand. The consensus sequence was calculated by the program PRETTY. While a vote weight of 0.5 was assigned to the two *P. involutus* phytases, all other genes were used with a vote weight of 1.0 for the consensus sequence calculation. At positions, where the program was not able to determine a consensus residue, the Basidio sequence contains a dash. Capitalized amino acid residues in the alignment at a given position belong to the amino acid coalition that establish the consensus residue.

Figure 5: Design of consensus phytase-10 amino acid sequence. Adding the phytase sequence of *Thermomyces lanuginosus* (Berka *et al.*, 1998) and the consensus sequence of the phytases from five *Basidiomycetes* to the alignment of Figure 2, an improved consensus sequence was calculated by the program PRETTY. Additionally, the amino acid sequence of *A. niger* T213 was omitted; therefore, a vote weight of 0.5 was used for the remaining *A. niger* phytase sequences. For further information see Example 8.

Figure 6: DNA and amino acid sequence of consensus phytase-10. The amino acid sequence is written above the corresponding DNA sequences using the one-letter code. The sequence of the oligonucleotides which were used to assemble the gene are in bold letters. The labels of oligonucleotides and the amino acids which were changed compared to those for consensus phytase -1 are underlined. The *fcp*10 gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, CP-7.10, CP-8.10, CP-9.10, CP-10.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10, CP-19.10, CP-20.10, CP-21.10, CP-22.10. The newly synthesized oligonucleotides are additionally marked by number 10. The phytase contains the following 32 exchanges relative to consensus phytase -1: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E. The mutations accentuated in bold letters revealed a stabilizing effect on consensus phytase-1 when tested as single mutations in consensus phytase-1.

Figure 7: Alignment for the design of consensus phytase-11. In contrast to the design of consensus phytase-10, for the design of the amino acid sequence of consensus phytase-11, all *Basidiomycete* phytases were used as independent sequences using an assigned vote weight of 0.2 for each *Basidiomycete* sequence. Additionally, the amino acid sequence of *A. niger* T213 phytase was used in that alignment, again.

Figure 8: DNA and amino acid sequence of consensus phytase-1-thermo[8]-Q50T-K91A. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

Figure 9: DNA and amino acid sequence of consensus phytase-10-thermo[3]-Q50T-K91A. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

Figure 10: DNA and amino acid sequence of *A. fumigatus* ATCC 13073 phytase α-mutant. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

Figure 11: DNA and amino acid sequence of consensus phytase-7. The amino acids are written above the corresponding DNA sequence using the one-letter code. The sequences of the oligonucleotides used to assemble the gene are in bold letters. Oligonucleotides and amino acids that were exchanged are underlined and their corresponding triplets are highlighted in small cases. The *fcp7* gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, CP-8.7, CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, CP-22. The newly synthesized oligonucleotides are additionally marked by number 7. The phytase contains the following 24 exchanges in comparison to the original consensus phytase -1: S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S.

Figure 12: Differential scanning calorimetry (DSC) of consensus phytase-1 and consensus phytase-10. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10 °C/min was applied up to 95 °C. DSC of consensus phytase-10 (upper graph) yielded a melting temperature of 85.4 °C, which is 7.3 °C higher than the melting point of consensus phytase-1 (78.1 °C, lower graph).

Figure 13: Differential scanning calorimetry (DSC) of consensus phytase-10-thermo[3]-Q50T and consensus phytase-10-thermo[3]-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10 °C/min was applied up to 95 °C. DSC of consensus phytase-10-thermo-[3]-Q50T (upper graph) yielded a melting temperature of 88.6 °C, while the melting point of consensus phytase-10-thermo[3]-Q50T-K91A was found at 89.3 °C.

Figure 14: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-10 and consensus phytase-10-thermo[3]-Q50T. For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 86 °C. The diluted supernatant of transformed *S. cerevisiae* strains was used for the determination. The other components of the supernatant showed no influence on the determination of the temperature optimum: △, consensus phytase-1; ◊, consensus phytase-10; ■, consensus phytase 10-thermo[3]-Q50T.

Figure 15: pH-dependent activity profile and substrate specificity of consensus phytase-10 and its variants thermo[3]-Q50T and thermo[3]-Q50T-K91A. Graph a) shows the pH-dependent activity profile of consensus phytase- 10 (□), consensus phytase-10-thermo[3]-Q50T (△), and consensus phytase-10-thermo[3]-Q50T-K91A (△).The phytase activity was determined using the standard assay in appropriate buffers (see Example 15) at different pH-values. Graph b) shows the corresponding substrate specificity tested by replacement of phytate by the indicated compounds in the standard assay; open bars, consensus phytase-10 (white bars, consensus phytase-10-thermo-Q50T; dark bars, consensus phytase-10-thermo-Q50T-K91A). The numbers correspond to the following compounds: 1, phytate; 2, *p*-nitrophenyl phosphate; 3, phenyl phosphate; 4, fructose-1,6-bisphosphate; 5, fructose-6-phosphate; 6, glucose-6-phosphate; 7, ribose-5-phosphate; 8, DL-glycerol-3-phosphate; 9, glycerol-2-phosphate; 10, 3-phosphoglycerate; 11, phosphoenolpyruvate; 12, AMP; 13, ADP; 14, ATP.

Figure 16: pH-dependent activity profile and substrate specificity of consensus phytase-1-thermo[8]-Q50T and of consensus phytase-1-thermo[8]-Q50T-K91A.. Graph a) shows the pH-dependent activity profile of the Q50T- (■) and the Q50T-K91A-variant (△).The phytase activity was determined using the standard assay in appropriate buffers (see Example 15) at different pH-values Graph b) shows the corresponding substrate specificities tested by replacement of phytate by the indicated compounds in the standard assay (open bars, consensus phytase-1-thermo[8]-Q50T; filled bars, consensus phytase-1-thermo[8]-Q50T-K91A). The substrates are listed in the legend of Figure 15.

Figure 17: Differential scanning calorimetry (DSC) of consensus phytase-1-thermo[8]-Q50T and consensus phytase-1-thermo[8]-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10 °C/min was applied up to 95 °C. DSC of consensus phytase-1-thermo[8]-Q50T (upper graph) showed a melting temperature of 84.7 °C, while the melting point of consensus phytase-1-thermo[8]-Q50T-K91A was found at 85.7 °C.

Figure 18: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-1-thermo[3] and consensus phytase-1-thermo[8]. For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 86 °C. Purified protein from the supernatant of transformed *S. cerevisiae* strains was used for the determination. O, consensus phytase-1;□, consensus

phytase-1-thermo [3]; △, consensus phytase-1-thermo[8].

Figure 19: Comparison of the pH-dependent activity profile and substrate specificity of consensus phytase-1, consensus phytase-7, and of the phytase from *A. niger* NRRL 3135.. Graph a) shows the pH-dependent activity profile of consensus phytase-1 (■), the phytase from *A. niger* NRRL 3135 (O), and of consensus phytase-7 (△).The phytase activity was determined using the standard assay in appropriate buffers (see Example 15) at different pH-values Graph b) shows the corresponding substrate specificity tested by replacement of phytate by the indicated compounds in the standard assay (black bars, *A. niger* NRRL 3135 phytase; grey bars, consensus phytase-1, dashed bars, consensus phytase-7). The substrates are listed in the legend of Figure 15.

Figure 20: Differential scanning calorimetry (DSC) of the phytase from A. fumigatus ATCC 13073 and of its stabilized α-mutant, which contains the following amino acid exchanges: F55Y, V100I, F114Y, A243L, S265P, N294D.

The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10 °C/min was applied up to 95 °C. DSC of consensus *A. fumigatus* 13073 phytase (lower graph) revealed a melting temperature of 62.5 °C, while the melting point of the α-mutant was found at 67.0 °C

Figure 21: Comparison of the temperature optimum of *A. fumigatus* 13073 wild-type phytase, its α-mutant, and a further stabilized α-mutant (E59A-S126N-R329H-S364T-G404A). For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 75 °C. The diluted supernatants of transformed *S. cerevisiae* strains were used for the determination. The other components of the supernatant showed no influence on the determination of the temperature optimum. O, *A. fumigatus* ATCC 13073 phytase; △, *A. fumigatus* ATCC 13073 α-mutant; □, *A. fumigatus* ATCC 13073 alpha-mutant-(E59A-S126N-R329H-S364T-G404A)-Q27T; ■ , *A. fumigatus* ATCC 13073 α-mutant-(E59A-S126N-R329H-S364T-G404A)-Q27T-K68A. The mutations Q51T and K92A in the *A. furnigatus* α-mutants correspond to -1 Q50T and K91A in consensus phytase, respectively.

Figure 22: Amino acid sequence of consensus phytase -12 (consphy12) which contains a number of active site residues transferred from the "basidio" consensus sequence to consensus phytase-10-thermo[3]-Q50T-K91A.

[0019] The culture medium used in the fermentation process in accordance with the present invention usually contains nutrients for the cells or microorganisms such as digestible nitrogen sources and inorganic substances, vitamins, micro- and trace elements and other growth-promoting factors. In addition, the culture medium contains a carbon source. Various organic or inorganic substances may be used as nitrogen sources in the fermentation process in accordance with the present invention, such as nitrates, ammonium salts, yeast extract, meat extract, peptone, casein, cornsteep liquor, amino acids and urea. Typical inorganic substances that can be used in the fermentation are calcium, iron, zinc, nickel, manganese, cobalt, copper, molybdenum, and alkali salts such as chlorides, sulphates and phosphates as well as boric acid. As a carbon source, glycerol or sugar-like mono-, di-, oligo- or polysaccharides, e.g., glucose, fructose, sucrose, maltose, starch, glycogen, cellulose or substrates containing such substances, e.g., molasses, glucose syrups and fructose syrups can be used. The concentration of glucose and / or methanol in the total feed stream may vary from about 10 to about 500 g/l for each component and is preferably from about 200 to about 300 g/l. While the fermentation medium is principally an aqueous medium such medium may contain organic solvents such as alcohols, e.g. methanol, ethanol or isopropanol. Further, the fermentation medium may also be a dispersion or suspension, in which case the fermentation is suitably carried out with stirring.

[0020] For continuous operation, the cells are optionally immobilized on a solid porous carrier. Any solid porous carrier with any porosity, size and geometry conventionally used in fermentation processes and exerting no toxic effects on the particular cell or microorganism which is to be immobilized can be used for the purpose of this invention. Examples of such carriers are those made from inorganic material and having a pore diameter of from about 0.5 to about 100 μm, preferably from about 10 to about 30 μm diameter. Examples of inorganic materials are ceramics and natural minerals such as steatite, zeolite, bentonite, silicates (glasses), aluminum silicates, aluminum oxide, magnesium aluminum silicates and magnesium aluminum oxides. Such carriers are commercially available, e.g., from Ceramtec, Marktredwitz, Germany, Schott Engineering GmbH, Mainz, Germany and others. Preferably, the carriers are spherical with a mean diameter of from about 0,2 to about 20 mm diameter. The carriers can be loaded with the living cells in a manner known per se by contacting the carrier particles with an appropriate cell culture. If desired, the carrier particles loaded with the cells can be further processed by applying a membrane-type coating layer, such as described in German Offenlegungsschrift DE 3421049. Suitably, the carrier is present in the fermentation vessel on a fixed bed. Further, the culture medium, its components and their containments, respectively are suitably sterilized prior to use if autosterilization (e.g., by methanol, ethanol, ammonia) cannot be guaranteed. Heat sterilization with steam (e.g., at 121°C and 1 bar pressure

during 20 minutes) and filtration (0.2 µm) for sensitive components are preferred. Alternative sterilization methods may be applied. Media components need not necessarily be sterilized when running the process in continuous mode.

[0021] Depending on the particular cell or organism used the fermentation may be carried out at a pH between about 2 and about 11. In a preferred aspect of the invention, the fermentation process for the manufacture of phytase is carried out using the microorganism, *Hansenula polymorpha* transformed by a phytase encoding DNA sequence as described in EP 897 010, EP 897 985, or Example 11 of the present case. According to that particular aspect of the invention, the preferred carbon source is a mixture of glucose and methanol. Further, in accordance with that particular aspect of the invention, the fermentation may be carried out at a pH betweeen about 4 and 5, preferably at about pH 4.6. A preferred temperature range for carrying out such fermentation process is between about 10 and 50 °C, more preferably the fermentation temperature is about 30 °C. The aeration rate is preferably adjusted to between about 0.01 and about 1.5 volume of gas per volume of liquid with a dissolved oxygen concentration (DO) of in between 0.01 and about 500 %. A DO of 100 % denotes oxygen saturation of the solution at atmospheric pressure (1 bar) and reactor temperature. The fermentation can be carried out at a pressure of from about 0.1 to about 100 bar, preferably, the fermentation is carried out at atmospheric pressure, i.e., at about 1 bar. The dilution rate can vary from about 0.001 to about 0.5 per hour.

[0022] The invention is illustrated further by the Examples given below.

Example 1

[0023] Storage solutions for feed medium were prepared as follows:

1.1 $CaCl_2/H_3BO_3$ Solution

[0024]

$CaCl_2 \cdot 2\ H_2O$	18.75	g/l
H_3BO_3	0.0125	g/l

[0025] This solution was sterilized at 121 °C for 20 minutes.

1.2 Microelements Solution

[0026]

$(NH_4)_2Fe(SO_4)_2 \cdot 6\ H_2O$	2.5	g/l
$CuSO_4 \cdot 5\ H_2O$	0.2	g/l
$ZnSO_4 \cdot 7\ H_2O$	0.75	g/l
$MnSO_4 \cdot 5\ H_2O$	1.0	g/l
Na-EDTA	2.5	g/l

[0027] This solution was sterilized at 121 °C for 20 minutes.

1.3 Trace Elements Solution

[0028]

$NiSO_4 \cdot 6\ H_2O$	0.025	g/l

(continued)

$CoCl_2 \cdot 6\ H_2O$	0.025	g/l
$Na_2MoO_4 \cdot 2\ H_2O$	0.025	g/l
KJ		

[0029] This solution was sterilized at 121 °C for 20 minutes.

1.4 Salts + Vitamin Solution

[0030]

KH_2PO_4	50.0	g/l
$NH_4H_2PO_4$	100.0	g/l
$MgSO_4 \cdot 7\ H_2O$	45.0	g/l
$(NH_4)_2SO_4$	50.0	g/l
KCl	23.0	g/l
NaCl	5.0	g/l
vitamin solution (D-biotin, 600 mg/l thiamin • HCl 200 g/l in 50 % isopropanol/water)	5.0	ml/l

[0031] The vitamin solution was sterilized by filtration (0.2 µm) and added to the salt solution that was sterilized at 121 °C for 20 minutes.

1.5 Glucose Solution

[0032] 770 g of D-glucose • H2O were dissolved in 480 g of water and sterilized (121 °C, 20 min) to yield 1 l solution containing 57 % (by weight) of D-glucose.

1.6 Methanol

[0033] Pure methanol was assumed to be sterile and filled into a sterilized flask.

1.7 Antifoam

[0034] A sterilized (121 °C, 20 min) solution of 10% antifoam (Struktol J 673, Schill & Seilacher, Hamburg, Germany) was provided for supply on demand by foam-control.

1.8 Base

[0035] A solution of ca. 12,5 % (by weight) of ammonia in sterile water was filled into a sterilized flask.

Example 2

[0036] A fixed bed bioreactor (1 litre) was set up following the principle illustrated in Figure 1 with individual storage flasks being provided for the solutions 1.1 to 1.8 of Example 1. The fixed bed of porous steatite spheres (4 mm diameter, pore diameter 10-30 µm, 280 pores per ml, CeramTec, Marktredwitz, Germany) was contained by a sieve plate at the top. The reactor was sterilized (121 °C, 20 min) and thereafter filled with an inoculum culture of *Hansenula polymorpha* transformed with a phytase encoding DNA as described, e.g. in EP 897 010, EP 897 985 or Example 11. Then the connection to the storage flasks was established. The inoculum culture was grown on a medium containing glycerol as a carbon source instead of glucose. The reactor was put to batch operation until all glycerol was consumed, which was determined by a rise of the dissolved oxygen concentration. Then the feed stream was turned on and the fermentation

was run under process conditions as given below:

Temperature	30	°C
pH	4.6	Diluted oxygen concentration
p_{total}	10^5	N/m^2
p_{O2}	10^5	N/m^2
Dilution rate	0.0067	h^{-1}
aeration rate	100	ml/min
V_{fluid}	1190	ml^{-1}
$V_{fixed\ bed}$	950	ml^{-1}

[0037] Substrate composition as provided by storage flasks 1-8; (actual concentrations in feed stream given) :

D-glucose	305	g/l
Methanol	264	g/l
$CaCl_2/H_3BO_3$ Solution	12.2	g/l
Microelement Solution	20.9	g/l
Trace Element Solution	17.2	g/l
Salts + vitamin Solution	44.7	g/l

Analytics:

[0038] Bio-Rad Protein Assay Kit I (Bio-Rad, Glattbrugg, Switzerland) was used to determine the total protein concentration. A factor for the calculation of phytase concentration (c_{phyt}) from total protein concentration (c_{tp}) was determined as $c_{phyt} = 0.76 \cdot c_{tp}$.

[0039] To determine the biomass in the medium two samples of 1 ml were centrifuged, washed with 1 ml of water, centrifuged again, dried at 85 °C for two days and weighed.

Results:

[0040] Under the above process conditions the biomass was 59 g/l. Given a dilution rate of 0.0067 per hour the productivity was 0.078 g of phytase per litre per hour.

[0041] In a fermentation that was run fed-batch-wise the biomass was 125 g/l; the productivity, however, was calculated to 0.054 g phytase per litre per hour.

Example 3

[0042] A fermentation in analogy to Example 2 but omitting the steatite spheres (i.e., without immobilisation of the microorganism) was carried out. A nutrient and a salt and vitamin solution of the following composition were pumped into the reactor separately:

Nutrient Solution:

[0043]

$NiSO_4 \cdot 6\ H_2O$	8.33	mg/l
$CoCl_2 \cdot 6\ H_2O$	8.33	mg/l
$Na_2MoO_4 \cdot 2\ H_2O$	8.33	mg/l
KJ	8.33	mg/l
$(NH_4)_2Fe(SO_4)_2 \cdot 6\ H_2O$	833.33	mg/l
$CuSO_4 \cdot 5\ H_2O$	66.67	mg/l
$ZnSO_4 \cdot 7\ H_2O$	250	mg/l
$MnSO_4 \cdot 5\ H_2O$	333.33	mg/l
Na-EDTA	833.33	mg/l
$CaCl_2 \cdot 2\ H_2O$	6250	mg/l
H_3BO_3	4.17	mg/l

Salts + Vitamins Solution:

[0044]

KH_2PO_4	50.0	g/l
$NH_4H_2PO_4$	100.0	g/l
$MgSO_4 \cdot 7\ H_2O$	45.0	g/l
$(NH_4)_2SO_4$	50.0	g/l
KCl	23.0	g/l
NaCl	5.0	g/l
vitamin solution (D-biotin, 600 mg/l thiamin • HCl 200 g/l in 50 % isopropanol/water)	5.0	ml/l

[0045] The supply of these two solutions was adjusted to provide in the feed stream a concentration of 51 g/l of Nutrient Solution and 61 g/l of Salts + Vitamins Solution. The dilution rate was adjusted to 0.009 h^{-1}. The pH was kept at 4.6 by addition of 12.5 wt% ammonium hydroxide.

[0046] Furthermore, Glucose Solution as in Example 1 and methanol were fed into the reactor separately to maintain a glucose concentration of 275 g/l and a methanol concentration of 260 g/l in the feed stream.

[0047] The productivity of this fermentation was 0.088 g phytase per litre per hour. Biomass in outflow was 58 g/l.

Example 4

[0048] In a fermentation process in analogy to Example 3 but adjusting glucose concentration to 290 g/l, methanol concentration to 260 g/l, and keeping the dilution rate constant at 0.009 h^{-1}, the productivity was 0.092 g phytase per litre per hour. Biomass in outflow was 60.4 g/l.

Example 5

[0049] In a fermentation process in analogy to Example 3 but adjusting glucose concentration to 270 g/l, methanol concentration to 280 g/l, and keeping the dilution rate constant at 0.009 h^{-1}, the productivity was 0.094 g phytase per

litre per hour. Biomass in outflow was 56.8 g/l.

Example 6:

Design of the amino acid sequence of consensus phytase-1

Alignment of the amino acid sequences

[0050] The alignment was calculated using the program PILEUP from the GCG Sequence Analysis Package Release 9.0 (Devereux *et al.*, 1984) with the standard parameters (gap creation penalty 12, gap extension penalty 4). The location of the gaps was refined using a text editor. Table 1 shows the sequences (see Figure 2), without the signal sequence, that were used for the performance of the alignment starting with the amino acid (aa) as mentioned in Table 1.

Table 1

Origin and vote weight of the phytase amino acid sequences used for the design of consensus phytase-1

- *phyA* from *Aspergillus terreus* 9A-1, aa 27, vote weight 0.5 (Mitchell *et al.*, 1997)
- *phyA* from *Aspergillus terreus* cbs116.46, aa 27, vote weight 0.5 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus niger* var. *awamori*, aa 27, vote weight 0.33 (Piddington *et al.*, 1993)
- *phyA* from *Aspergillus niger* T213, aa 27, vote weight 0.33
- *phyA* from *Aspergillus niger* strain NRRL3135, aa 27, vote weight 0.33 (van Hartingsveldt *et al.*, 1993)
- *phyA* from *Aspergillus fumigatus* ATCC 13073, aa 26, vote weight 0.2 (Pasamontes *et al.*, 1997)
- *phyA* from *Aspergillus fumigatus* ATCC 32722, aa 26, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus fumigatus* ATCC 58128, aa 26, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus fumigatus* ATCC 26906, aa 26, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus fumigatus* ATCC 32239, aa 30, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Emericella nidulans* , aa 25, vote weight 1.0 (Pasamontes *et al.*, 1997a)
- *phyA* from *Talaromyces thermophilus* ATCC 20186, aa 24, vote weight 1.0 (Pasamontes *et al.*, 1997a)
- *phyA* from *Myceliophthora thermophila*, aa 19, vote weight 1.0 (Mitchell *et al.*, 1997)

Calculation of the amino acid sequence of consensus phytase-1

[0051] Using the refined alignment as input, the consensus sequence was calculated by the program PRETTY from the GCG Sequence Analysis Package Release 9.0 (Devereux *et al.*, 1984). PRETTY prints sequences with their columns aligned and can display a consensus sequence for an alignment. A vote weight that pays regard to the similarity between the amino acid sequences of the aligned phytases was assigned to all sequences. The vote weight was set in such a way that the combined impact of all phytases from one sequence subgroup (same species, but from different strains), e. g. the amino acid sequences of all phytases from *A. fumigatus*, on the election was set one, that means that each sequence contributes with a value of 1 divided by the number of strain sequences (see Table 1). By this means, it was possible to prevent that very similar amino acid sequences, e. g. of the phytases from different *A. fumigatus* strains, dominate the calculated consensus sequence.

[0052] The program PRETTY was started with the following parameters: The plurality defining the number of votes below which there is no consensus was set on 2.0. The threshold, which determines the scoring matrix value below which an amino acid residue may not vote for a coalition of residues, was set on 2. PRETTY used the PrettyPep.Cmp consensus scoring matrix for peptides.

[0053] Ten positions of the alignment (position 46, 66, 82, 138, 162, 236, 276, 279, 280, 308; Figure 2), for which the program was not able to determine a consensus residue, were filled by hand according to the following rules: if a most frequent residue existed, this residue was chosen (138, 236, 280); if a prevalent group of similar equivalent residues occurred, the most frequent or, if not available, one residue of this group was selected (46, 66, 82, 162, 276, 308). If there was neither a prevalent residue nor a prevalent group, one of the occurring residues was chosen according to

common assumptions on their influence on the protein stability (279). Eight other positions (132, 170, 204, 211, 275, 317, 384, 447; Figure 2) were not filled with the amino acid residue selected by the program but normally with amino acids that occur with the same frequency as the residues that were chosen by the program. In most cases, the slight underrating of the three *A. niger* sequences (sum of the vote weights: 0.99) was eliminated by this correction.

Conversion of the consensus phytase-1 amino acid sequence to a DNA sequence

[0054] The first 26 amino acid residues of *A. terreus* cbs116.46 phytase were used as signal peptide and, therefore, fused to the N-terminus of all consensus phytases. For this stretch, we used a special method to calculate the corresponding DNA sequence. Purvis et al (1987) proposed that the incorporation of rare codons in a gene has an influence on the folding efficiency of the protein. The DNA sequence for the signal sequence was calculated using the approach of Purvis et al (1987) and optimized for expression in *S. cerevisiae*. For the remaining parts of the protein, we used the codon frequency table of highly expressed *S. cerevisiae* genes, obtained from the GCG program package, to translate the calculated amino acid sequence into a DNA sequence.

[0055] The resulting sequence of the *fcp* gene is shown in Figure 3.

Construction and cloning of the consensus phytase-1 gene

[0056] The calculated DNA sequence of consensus phrase-1 (*fcp*) was divided into oligonucleotides of 85 bp, alternately using the sequence of the sense and the anti-sense strand. Every oligonucleotide overlaps 20 bp with its previous and its following oligonucleotide of the opposite strand. The location of all primers, purchased from Microsynth, Balgach (Switzerland) and obtained in a PAGE-purified form, is indicated in Figure 3.

PCR-Reactions

[0057] In three PCR reactions, the synthesized oligonucleotides were composed to the entire gene. For the PCR, the High Fidelity Kit from Boehringer Mannheim (Boehringer Mannheim, Germany) and the thermo cycler The Protokol (TM) from AMS Biotechnology (Europe) Ltd. (Lugano, Switzerland) were used.

[0058] Oligonucleotides CP-1 to CP-10 (Mix 1, Figure 3) were mixed to a concentration of 0.2 pmol/µl of each oligonucleotide. A second oligonucleotide mixture (Mix 2) was prepared with CP-9 to CP-22 (0.2 pmol/µl of each oligonucleotide). Additionally, four short primers were used in the PCR reactions:

CP-a: *Eco* RI

5'-TATAT*GAATTC*ATGGGCGTGTTCGTC-3' (SEQ ID No. 1)

CP-b:

5'-TGAAAAGTTCATTGAAGGTTTC-3' (SEQ ID No. 2)

CP-c:

5'-TCTTCGAAAGCAGTACAAGTAC-3' (SEQ ID No. 3)

CP-e: *Eco* RI

5'-TATAT*GAATTC*TTAAGCGAAAC-3' (SEQ ID No. 4)

PCR reaction *a*:

10 µl Mix 1 (2.0 pmol of each oligonucleotide)
2 µl nucleotides (10 mM each nucleotide)
2 µl primer CP-a (10 pmol/µl)
2 µl primer CP-c (10 pmol/µl)

	10,0 µl PCR buffer 0.75 µl polymerase mixture (2.6U) 73.25 µl H_2O
PCR reaction *b*:	10 µl Mix 2 (2.0 pmol of each oligonucleotide) 2 µl nucleotides (10 mM each nucleotide) 2 µl primer CP-b (10 pmol/µl) 2 µl primer CP-e (10 pmol/µl) 10,0 µl PCR buffer 0.75 µl polymerase mixture (2.6 U) 73.25 µl H_2O
Reaction conditions for PCR reactions *a* and *b*:	step 1 2 min - 45°C step 2 30 sec - 72°C step 3 30 sec - 94°C step 4 30 sec - 52°C step 5 1 min - 72°C

[0059] Steps 3 to 5 were repeated 40-times.

[0060] The PCR products (670 and 905 bp) were purified by an agarose gel electrophoresis (0.9% agarose) and a following gel extraction (QIAEX II Gel Extraction Kit, Qiagen, Hilden, Germany). The purified DNA fragments were used for the PCR reaction *c*.

PCR reaction *c*:	6 µl PCR product of reaction a (≈50 ng) 6 µl PCR product of reaction b (≈50 ng) 2 µl primer CP-a (10 pmol/µl) 2 µl primer CP-e (10 pmol/µl) 10,0 µl PCR buffer 0.75 µl polymerase mixture (2.6 U) 73.25 µl H_2O
Reaction conditions for PCR reaction *c*:	step 1 2 min - 94°C step 2 30 sec - 94°C step 3 30 sec - 55°C step 4 1 min - 72°C

[0061] Steps 2 to 4 were repeated 31-times.

[0062] The resulting PCR product (1.4 kb) was purified as mentioned above, digested with *Eco* RI, and ligated in an *Eco* RI-digested and dephosphorylated pBsk(-)-vector (Stratagene, La Jolla, CA, USA). 1 µl of the ligation mixture was used to transform *E. coli* XL-1 competent cells (Stratagene, La Jolla, CA, USA). All standard procedures were carried out as described by Sambrook *et al.* (1987). The DNA sequence of the constructed consensus phytase gene (*fcp*, Figure 3) was controlled by sequencing as known in the art.

Example 7

Design of an improved consensus phytase (consensus phytase-10) amino acid sequence

[0063] The alignments used for the design of consensus phytase-10 were calculated using the program PILEUP from the GCG Sequence Analysis Package Release 9.0 (Devereux *et al.*, 1984) with the standard parameters (gap creation penalty 12, gap extension penalty 4). The location of the gaps was refined using a text editor.

[0064] The following sequences were used for the alignment of the *Basiodiomycete* phytases starting with the amino acid (aa) mentioned in Table 2:

Table 2

Origin and vote weight of five *Basidiomycete* phytases used for the calculation of the corresponding amino acid consensus sequence (basidio)
- *phyA1* from *Paxillus involutus* NN005693, aa 21, vote weight 0.5 (WO 98/28409)
- *phyA2* from *Paxillus involutus* NN005693, aa 21, vote weight 0.5 (WO 98/28409)
- *phyA* from *Trametes pubescens* NN9343, aa 24, vote weight 1.0 (WO 98/28409)
- *phyA* from *Agrocybe pediades* NN009289, aa 19, vote weight 1.0 (WO 98/28409)
- *phyA* from *Peniophora lycii* NN006113, aa 21, vote weight 1.0 (WO 98/28409)

[0065] The alignment is shown in Figure 4.

[0066] In Table 3 the genes, which were used for the performance of the final alignment, are arranged. The first amino acid (aa) of the sequence which is used in the alignment is mentioned behind the organism's designation.

Table 3

Origin and vote weight of the phytase sequences used for the design of consensus phytase 10
- *phyA* from *Aspergillus terreus* 9A-1, aa 27, vote weight 0.5 (Mitchell *et al.*, 1997)
- *phyA* from *Aspergillus terreus* cbs116.46, aa 27, vote weight 0.5 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus niger* var. *awamori*, aa 27, vote weight 0.5 (Piddington *et al.*, 1993)
- *phyA* from *Aspergillus niger* strain NRRL3135, aa 27, vote weight 0.5 (van Hartingsveldt *et al.*, 1993)
- *phyA* from *Aspergillus fumigatus* ATCC 13073, aa 26, vote weight 0.2 (Pasamontes *et al.*, 1997)
- *phyA* from *Aspergillus fumigatus* ATCC 32722, aa 26, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus fumigatus* ATCC 58128, aa 26, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus fumigatus* ATCC 26906, aa 26, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Aspergillus fumigatus* ATCC 32239, aa 30, vote weight 0.2 (EP 897 985; Figur 1)
- *phyA* from *Emericella nidulans* , aa 25, vote weight 1.0 (Pasamontes *et al.*, 1997a)
- *phyA* from *Talaromyces thermophilus* ATCC 20186, aa 24, vote weight 1.0 (Pasamontes *et al.*, 1997a)
- *phyA* from *Myceliophthora thermophila*, aa 19, vote weight 1.0 (Mitchell *et al.*, 1997)
- *phyA* from *Thermomyces lanuginosa*, aa 36, vote weight 1.0 (Berka *et al.*, 1998)
- Consensus sequence of five *Basidiomycete* phytases, vote weight 1.0 (Basidio, Figure 4)

[0067] The corresponding alignment is shown in Figure 5.

Calculation of the amino acid sequence of consensus phytase-10

[0068] To improve the alignment, we combined the consensus sequence of five phytases from four different *Basidiomycetes*, called Basidio, still containing the undefined sequence positions (see Figure 4), nearly all phytase sequences used for calculation of the original consensus phytase and one new phytase sequence from the *Ascomycete Thermomyces lanuginosus* to a larger alignment.

We set plurality on 2.0 and threshold on 3. The used vote weights are listed in Table 3. The alignment and the corresponding consensus sequence are presented in Figure 5. The new consensus phytase -10 sequence has 32 different amino acids in comparison to the original consensus phytase. Positions for which the program PRETTY was not able to calculate a consensus amino acid residue were filled according to rules mentioned in Example 6. None of the residues suggested by the program was replaced.

[0069] Furthermore, we included all Basidiomycete phytases as single amino acid sequences but assigning a vote weight of 0.2 in the alignment. The corresponding alignment is shown in Figure 7. The calculated consensus amino acid

sequence (consensus phytase-11) has the following differences to the sequence of consensus phytase-10: D35X, X(K)69K, X(E)100E, A101R, Q134N, X(K)153N, X(H)190H, X(A)204S, X(E)220D, E222T, V227A, X(R)271R, H287A, X(D)288D, X(K)379K, X(I)389I, E390X, X(E)415E, X(A)416A, X(R)446L, E463A, where the numbering is as in Fig. 6.

[0070] Letter X means that the program was not able to calculate a consensus amino acid; the amino acid in parenthesis corresponds to the amino acid finally included into the consensus phytase-10.

[0071] We also checked single amino acid replacements suggested by the improved consensus phytase sequences 10 and 11 on their influence on the stability of the original consensus phytase -1. The approach is described in example 8.

Conversion of consensus phytase-10 amino acid sequence to a DNA sequence

[0072] The first 26 amino acid residues of *A. terreus* cbs116.46 phytase were used as signal peptide and, therefore, fused to the *N*-terminus of consensus phytase-10. The used procedure is further described in Example 6.

[0073] The resulting sequence of the *fcp*10 gene is shown in Figure 6.

Construction and cloning of the consensus phytase-10 gene (*fcp*10)

[0074] The calculated DNA sequence of *fcp*10 was divided into oligonucleotides of 85 bp, alternately using the sequence of the sense and the anti-sense strand. Every oligonucleotide overlaps 20 bp with its previous and its following oligonucleotide of the opposite strand. The location of all primers, purchased from Microsynth, Balgach (Switzerland) and obtained in a PAGE-purified form, is indicated in Figure 6.

PCR-Reactions

[0075] In three PCR reactions, the synthesized oligonucleotides were composed to the entire gene. For the PCR, the High Fidelity Kit from Boehringer Mannheim (Boehringer Mannheim, Mannheim, Germany) and the thermocycler The Protokol™ from AMS Biotechnology (Europe) Ltd. (Lugano, Switzerland) were used. The following oligonucleotides were used in a concentration of 0.2 pmol/ml.

Mix 1.10: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, CP-7.10, CP-8.10, CP-9.10, CP-10.10

Mix 2.10: CP-9.10, CP-10.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP-18.10, CP-19.10, CP-20.10, CP-21.10, CP-22.10

[0076] The newly synthesized oligonucleotides are marked by number 10. The phytase contains the following 32 exchanges, which are underlined in Figure 6, in comparison to the original consensus phytase -1: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E.

[0077] Four short PCR primers were used for the assembling of the oligonucleotides:

CP-a: *Eco* RI
5'-TATAT*GAATTC*ATGGGCGTGTTCGTC-3' (SEQ, ID No. 1)

CP-b:
5'-TGAAAAGTTCATTGAAGGTTTC-3' (SEQ, ID No. 2)

CP-c.10:
5'-TCTTCGAAAGCAGTACACAAAC-3' (SEQ, ID No. 5)

CP-e: *Eco* RI
5'-TATAT*GAATTC*TTAAGCGAAAC-3' (SEQ, ID No. 4)

PCR reaction *a*:	10 µl Mix 1.10 (2.0 pmol of each oligonucleotide) 2 µl nucleotides (10 mM each nucleotide) 2 µl primer CP-a (10 pmol/ml) 2 µl primer CP-c.10 (10 pmol/ml) 10,0 µl PCR buffer 0.75 µl polymerase mixture (2.6 U) 73.25 µl H_2O
PCR reaction *b*:	10 µl Mix 2.10 (2.0 pmol of each oligonucleotide) 2 µl nucleotides (10 mM each nucleotide) 2 µl primer CP-b (10 pmol/ml) 2 µl primer CP-e (10 pmol/ml) 10,0 µl PCR buffer 0.75 µl polymerase mixture (2.6 U) 73.25 µl H_2O
Reaction conditions for PCR reactions *a* and *b*:	step 1 2 min - 45°C step 2 30 sec - 72 °C step 3 30 sec - 94 °C step 4 30 sec - 52 °C step 5 1 min - 72°C

[0078] Steps 3 to 5 were repeated 40-times.

[0079] The PCR products (670 and 905 bp) were purified by an agarose gel electrophoresis (0.9% agarose) and a following gel extraction (QIAEX II Gel Extraction Kit, Qiagen, Hilden, Germany). The purified DNA fragments were used for the PCR reaction *c*.

PCR reaction *c*:	6 µl PCR product of reaction a(≈50 ng) 6 µl PCR product of reaction b(≈50 ng) 2 µl primer CP-a (10 pmol/ml) 2 µl primer CP-e (10 pmol/ml) 10,0 µl PCR buffer 0.75 µl polymerase mixture (2.6 U) 73.25 µl H_2O
Reaction conditions for PCR reaction *c*:	step 1 2 min - 94°C step 2 30 sec - 94 °C step 3 30 sec - 55 °C step 4 1 min - 72°C

Steps 2 to 4 were repeated 31-times.

[0080] The resulting PCR product (1.4 kb) was purified as mentioned above, digested with *Eco* RI, and ligated in an *Eco* RI-digested and dephosphorylated pBsk(-)-vector (Stratagene, La Jolla, CA, USA). 1 µl of the ligation mixture was used to transform *E. coli* XL-1 competent cells (Stratagene, La Jolla, CA, USA). All standard procedures were carried out as described by Sambrook *et al.* (1987). The DNA sequence of the constructed gene (*fcp10*) was checked by sequencing as known in the art.

Example 8

Increasing the thermostability of consensus phytase-1 by introduction of single mutations suggested by the amino acid sequence of consensus phytase-10 and/or consensus phytase-11

[0081] In order to increase the thermostability of homologous genes, it is also possible to test the stability effect of each differing amino acid residue between the protein of interest and the calculated consensus sequence and to combine all stabilizing mutations into the protein of interest. We used the consensus phytase -1 as protein of interest and tested the effect on the protein stability of 34 amino acids, which differed between consensus phytase -1 on one hand and consensus phytases 10 and/or -11 on the other hand, by single mutation..

[0082] To construct muteins for expression in *A. niger*, *S. cerevisiae*, or *H. polymorpha*, the corresponding expression plasmid containing the consensus phytase gene was used as template for site-directed mutagenesis (see Examples 11 - 13). Mutations were introduced using the "quick exchange™ site-directed mutagenesis kit" from Stratagene (La Jolla, CA, USA) following the manufacturer's protocol and using the corresponding primers. All mutations made and their corresponding primers are summarized in Table 4. Plasmids harboring the desired mutation were identified by DNA sequence analysis as known in the art.

Table 4: Primers used for site-directed mutagenesis of consensus phytases

(Exchanged bases are highlighted in bold. The introduction of a restriction site is marked above the sequence. When a restriction site is written in parenthesis, the mentioned site was destroyed by introduction of the mutation.)

mutation	Primer set
	Kpn I
Q50T	5'-CACTTGTGG***GGTACC***TACTCTCCATACTTCTC-3' (SEQ ID No. 6)
	5'-GAGAAGTATGGAGAGTA***GGTACC***CCACAAGTG-3' (SEQ ID No. 7)
Y54F	5'-GGTCAATACTCTCCATTCTTCTCTTTGGAAG-3' (SEQ ID No. 8)
	5'-CTTCCAAAGAGAAGAATGGAGAGTATTGACC-3' (SEQ ID No. 9)
E58A	5'-CATACTTCTCTTTGGCAGACGAATCTGC-3' (SEQ ID No. 10)
	5'-GCAGATTCGTCTGCCAAAGAGAAGTATG-3' (SEQ ID No. 11)

Aat II

D69K 5'-CTCCA*GACGTC*CCAAAGGACTGTAGAGTTAC-3' (SEQ ID No. 12)
5'-GTAACTCTACAGTCCTTTGGG*ACGTC*TGGAG-3' (SEQ ID No. 13)

Aat II

D70G 5'-CTCCA*GACGTC*CCAGACGGCTGTAGAGTTAC-3' (SEQ ID No. 14)
5'-GTAACTCTACAGCCGTCTGG*GACGTC*TGGAG-3' (SEQ ID No. 15)

K91A 5'-GATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTG-3'
(SEQ ID No. 16)
5'-CAGAGTAAGCCTTAGACGCAGAAGAAGTTGGGTATC-3'
(SEQ ID No. 17)

Sca I

A94K 5'-CTTCTAAGTCTAAG*AAGTACT*CTGCTTTG-3' (SEQ ID No. 18)
5'-CAAAGCAG*AGTACTT*CTTAGACTTAGAAG-3'(SEQ ID No. 19)

A101R 5'-GCTTACTCTGCTTTGATTGAACGGATTCAAAAGAACGCTAC-3'

5'-GTAGCGTTCTTTTGAATCCGTTCAATCAAAGCAGAGTAAGC-3'

N134Q 5'-CCATTCGGTGAACAGCAAATGGTTAACTC-3' (SEQ ID No. 22)
5'-GAGTTAACCATTTGCTGTTCACCGAATGG-3' (SEQ ID No. 23)

Nru I

K153N 5'-GATACAAGGCTC*TCGCGA*GAAACATTGTTC –3' (SEQ ID No. 24).
5'-GGAACAATGTTTC*TCGCGA*GAGCCTTGTATC-3' (SEQ ID No. 25)

Bss HI

I158V 5'-GATTGTTCCATTCGT*GCGCGC*TTCTGGTTC-3' (SEQ ID No. 26)
5'-GAACCAGAA*GCGCGC*ACGAATGGAACAATC-3' (SEQ ID No. 27)

Bcl I

D197N 5'-CTCCAGTTATTAACG*TGATCA*TTCCAGAAGG-3' (SEQ ID No. 28)
5'-CCTTCTGGAA*TGATCA*CGTTAATAACTGGAG-3' (SEQ ID No. 29)

Apa I

S187A 5'-GGCTGACCCAGG*GGCCC*AACCACACCAAGC-3' (SEQ ID No. 30)
5'-GCTTGGTGTGGTT*GGGCCC*CTGGGTCAGCC-3' (SEQ ID No. 31)

Nco I

T214L 5'-CACTTTGGA*CCATGG*TCTTTGTACTGCTTTCG-3' (SEQ ID No. 32)
5'-CGAAAGCAGTACAAAGA*CCATGG*TCCAAAGTG-3' (SEQ ID No. 33)

Avr II

E222T 5'-GCTTTCGAAGACTCTA*CCCTAGG*TGACGACGTTG-3' (SEQ ID No. 34)
5'-CAACGTCGTCA*CCTAGG*GTAGAGTCTTCGAAAGC-3' (SEQ ID No. 35)

V227A 5'-GGTGACGACGCTGAAGCTAACTTCAC-3' (SEQ ID No. 36)
5'-GTGAAGTTAGCTTCAGCGTCGTCACC-3'(SEQ ID No. 37)

Sac II
L234V 5'-CTAACTTCA*CCGCGG*TGTTCGCTCCAG-3' (SEQ ID No. 38)
5'-CTGGAGCGAACACCGCGGTGAAGTTAG-3' (SEQ ID No. 39)

A238P 5'-GCTTTGTTCGCTCCACCTATTAGAGCTAGATTGG-3' (SEQ ID No. 40)
5'-CCAATCTAGCTCTAATAGGTGGAGCGAACAAAGC-3' (SEQ ID No. 41)

Hpa I
T251N 5'-GCCAGGT*GTTAAC*TTGACTGACGAAG-3' (SEQ ID No. 42)
5'-TTCGTCAGTCAA*GTTAAC*ACCTGGC-3' (SEQ ID No. 43)

Aat II
Y259N 5'-GACGAA*GACGTC*GTTAACTTGATGGAC-3' (SEQ ID No. 44)
5'-GTCCATCAAGTTAAC*GACGTC*TTCGTC-3' (SEQ ID No. 45)

Asp I
E267D 5'-GTCCATTC*GACACTGTC*GCTAGAACTT C-3' (SEQ ID No. 46)
5'-GAAGTTCTAGC*GACAGTGTC*GAATGGAC-3' (SEQ ID No. 47)

E277Q 5'-CTGACGCTACTCAGCTGTCTCCATTC-3' (SEQ ID No. 48)
5'-GAATGGAGACAGCTGAGTAGCGTCAG-3' (SEQ ID No. 49)

A283D 5'-GTCTCCATTCTGTGATTTGTTCACTCAC-3' (SEQ ID No. 50)
5'-GTGAGTGAACAAATCACAGAATGGAGAC-3' (SEQ ID No. 51)

Ksp I
H287A 5'-GCTTTGTTCA*CCGCGG*ACGAATGGAG-3' (SEQ ID No. 52)
5'-CTCCATTCGT*CCGCGG*TGAACAAAGC-3' (SEQ ID No. 53)

Bam HI
R291I 5'-CACGACGAATG*GATCC*AATACGACTAC-3' (SEQ ID No. 54)
5'-GTAGTCGTATT*GGATCC*ATTCGTCGTG-3' (SEQ ID No. 55)

Bsi WI
Q292A 5'-GACGAATGGAGAG*CGTACG*ACTACTTG-3' (SEQ ID No. 56)
5'-CAAGTAGT*CGTACG*CTCTCCATTCGTC-3' (SEQ ID No. 57)

Hpa I
A320V 5'-GGTGTTGGTTTC*GTTAAC*GAATTGATTGC-3' (SEQ ID No. 58)
5'-GCAATCAATTC*GTTAAC*GAAACCAACACC-3' (SEQ ID No. 59)

(Bgl II)

R329H 5'-GCTAGATTGACT*CACTCT*CCAGTTCAAG-3' (SEQ ID No. 60)
5'-CTTGAACTGGAGA*GTGAGT*CAATCTAGC-3' (SEQ ID No. 61)

Eco RV

S364T 5'-CTCACGACAACACTAT*GATATC*TATTTTCTTC-3' (SEQ ID No. 62)
5'-GAAGAAAATA*GATATC*ATAGTGTTGTCGTGAG-3' (SEQ ID No. 63)

Nco I

I366V 5'-CGACAACT*CCATGG*TTTCTATTTTCTTCGC-3' (SEQ ID No. 64)
5'-GCGAAGAAAATAGAAA*CCATGG*AGTTGTCG-3' (SEQ ID No. 65)

Kpn I

A379K 5'-GTACAAC*GGTACC*AAGCCATTGTCTAC-3' (SEQ ID No. 66)
5'-GTAGACAATGGCTT*GGTACC*GTTGTAC-3' (SEQ ID No. 67)

S396A 5'-CTGACGGTTACGCTGCTTCTTGGAC-3' (SEQ ID No. 68
5'-GTCCAAGAAGCAGCGTAACCGTCAG-3' (SEQ ID No. 69)

G404A 5'-CTGTTCCATTCGCTGCTAGAGCTTAC-3' (SEQ ID No. 70)
5'-GTAAGCTCTAGCAGCGAATGGAACAG-3' (SEQ ID No. 71)

Q415E 5'-GATGCAATGTGAAGCTGAAAAGGAACC-3' (SEQ ID No. 72)
5'-GGTTCCTTTTCAGCTTCACATTGCATC-3' (SEQ ID No. 73)

Sal I

A437G 5'-CACGGTTGTGGT*GTCGAC*AAGTTGGG-3' (SEQ ID No. 74)
5'-CCCAACTT*GTCGAC*ACCACAACCGTG-3' (SEQ ID No. 75)

Mun I

A463E 5'-GATCTGGTGG*CAATTG*GGAGGAATGTTTCG-3' (SEQ ID No. 76)
5'-CGAAACATTCCTCC*CAATTG*CCACCAGATC-3' (SEQ ID No. 77)

and accordingly for other mutations.

[0083] The temperature optimum of the purified phytases, expressed in *Saccharomyces cerevisiae* (Example 14), was determined as outlined in Example 14. Table 5 shows the effect on the stability of consensus phytase -1 for each mutation introduced.

Table 5: Stability effect of the individual amino acid replacements in consensus phytase-1

(+ or - means a positive, respectively, negative effect on the protein stability up to 1 °C, ++ and -- means a positive, respectively, negative effect on the protein stability between 1 and

3 °C; the number 10 or 11 corresponds to the consensus phytase sequence that suggested the amino acid replacement.)

stabilizing		neutral		destabilizing	
mutation	effect	mutation	effect	mutation	effect
E58A (10)	+	D69A	±	Y54F (10)	-
D69K (11)	+	D70G (10)	±	V73I	-
D197N (10)	+	N134Q (10)	±	A94K (10)	-
T214L (10)	+ +	G186H	±	A101R (11)	-
E222T (11)	+ +	S187A (10)	±	K153N (11)	-
E267D (10)	+	T214V	±	I158V (10)	- -
R291I*	+	T251N (10)	±	G203A	- -
R329H (10)	+	Y259N (10)	±	G205S	-
S364T (10)	+ +	A283D (10)	±	A217V	-
A379K (11)	+	A320V (10)	±	V227A (11)	- -
G404A (10)	+ +	K445T	±	L234V (10)	-
		A463E (10)	±	A238P (10)	- -
				E277Q (10)	-
				H287A (11)	-
				Q292A (10)	-
				I366V (10)	-
				S396A (10)	- -
				Q415E (11)	-
				A437G (10)	- -
				E451R	- -

*: This amino acid replacement was found in another round of mutations.

[0084] We combined eight positive mutations (E58A, D197N, E267D, R291I, R329H, S364T, A379K, G404A) in consensus phytase -1 using the primers and the technique mentioned above in this example. Furthermore, the mutations Q50T and K91A were introduced which mainly influence the catalytical characteristics of the phytase (see EP 897

985 as well as Example 14). The DNA and amino acid sequence of the resulting phytase gene (consensus phytase-1-thermo[8]-Q50T-K91A) is shown in Figure 8. In this way, the temperature optimum and the melting point of the consensus phytase was increased by 7 ° C (Figure 16, 17, 18).

[0085] Using the results of Table 5, we further improved the thermostability of consensus phytase 10 by the following back mutations K94A, V158I, and A396S that revealed a strong negative influence on the stability of consensus phytase -1. The resulting protein is consensus phytase-10-thermo [3]. Furthermore, we introduced the mutations Q50T and K91A which mainly influence the catalytical characteristics of consensus phytase (see EP 897 485 as well as Example 14 and Figures 15 and 16). The resulting DNA and amino acid sequence is shown in Figure 9. The optimized phytase showed a 4 °C higher temperature optimum and melting point than consensus phytase -10 (Figures 13 and 14). Furthermore, the phytase has also a strongly increased specific activity with phytate as substrate of 250 U/mg at pH 5.5 (Figure 15).

Example 9

Stabilization of the phytase of *A. fumigatus* ATCC 13073 by replacement of amino acid residues with the corresponding consensus phytase-1 and consensus phytase-10 residues

[0086] At six typical positions where the *A. fumigatus* 13073 phytase is the only or nearly the only phytase in the alignment of Figure 2 that does not contain the corresponding consensus phytase amino acid residue, the non-consensus amino acid residue was replaced by the consensus one. In a first round, the following amino acids were substituted in *A. fumigatus* 13073 phytase, containing the Q51T substitution and the signal sequence of *A. terreus* cbs.116.46 phytase (see Figure 10):

F55(28)Y, V100(73)I, F114(87)Y, A243(220)L, S265(242)P, N294(282)D.

[0087] The numbers in parentheses refer to the numbering of Figure 2.

[0088] In a second round, four of the seven stabilizing amino acid exchanges (E59A, R329H, S364T, G404A) found in the consensus phytase-10 sequence and, tested as single mutations in consensus phytase-1 (Table 5), were additionally introduced into the *A. fumigatus* α-mutant. Furthermore, the amino acid replacement S154N, shown to reduce the protease susceptibility of the phytase, was introduced.

[0089] The mutations were introduced as described in example 8 (see Table 6) and expressed as described in example 11 to 13. The resulting *A. fumigatus* 13073 phytase variants were called a-mutant and α-mutant-E59A-S154N-R329H-S364T-G404A.

[0090] The temperature optimum (60 °C, Figure 21) and the melting point (67.0 °C, Figure 20) of the *A. fumigatus* 13073 phytase α-mutant were increased by 5 - 7°C in comparison to the values of the wild-type (temperature optimum: 55 °C, T_m: 60 °C). The five additional amino acid replacements further increased the temperature optimum by 3 °C (Figure 21).

Table 6: Mutagenesis primers for stabilization of *A. fumigatus* phytase ATCC 13073

Mutation	Primer
F55Y	5'-CACGTACTCGCCATACTTTTCGCTCGAG-3' (SEQ ID No. 78)
	5'-CTCGAGCGAAAAGTATGGCGAGTACGTG-3' (SEQ ID No. 79)
	(*Xho* I)
E58A	5'-CCATACTTTTCG*CTCGCG*GACGAGCTGTCCGTG-3' (SEQ ID NO. 80)
	5'-CACGGACAGCTCGTC*CGCGAG*CGAAAAGTAGG-3' (SEQ ID NO. 81)
V100I	5'-GTATAAGAAGCTTATTACGGCGATCCAGGCC-3' (SEQ ID No. 82)
	5'-GGCCTGGATCGCCGTAATAAGCTTCTTATAC-3' (SEQ ID No. 83)
F114Y	5'-CTTCAAGGGCAAGTACGCCTTTTTGAAGACG-3' (SEQ ID No. 84)
	5'-CGTCTTCAAAAAGGCGTACTTGCCCTTGAAG-3' (SEQ ID No. 85)
A243L	5'-CATCCGAGCTCGCCTCGAGAAGCATCTTC-3' (SEQ ID No. 86)
	5'-GAAGATGCTTCTCGAGGCGAGCTCGGATG-3' (SEQ ID No. 87)
S265P	5'-CTAATGGA TGTGTCCGTTTGATACGGTAG-3' (SEQ ID No. 88)
	5'-CTACCGTATCAAACGGACACATGTCCATTAG-3' (SEQ ID No. 89)

N294D 5'-GTGGAAGAAGTACGACTACCTTCAGTC-3' (SEQ ID No. 90)
5'-GACTGAAGGTAGTCGTACTTCTTCCAC-3' (SEQ ID No. 91)

(*Mlu* I)

R329H 5'-GCCCGGTTG*ACGCAT*TCGCCAGTGCAGG-3' (SEQ ID No. 92)
5'-CCTGCACTGGCGA*ATGCGT*CAACCGGGC-3' (SEQ ID No. 93)

Nco I

S364T 5'-CACACGACAACA*CCATGG*TTTCCATCTTC-3' (SEQ ID No. 94)
5'-GAAGATGGAAACCATGGTGTTGTCGTGTG-3' (SEQ ID No. 95)

(*Bss* HI)

G404A 5'-GTGGTGCCTTTCG*CCGCGC*GAGCCTACTTC-3' (SEQ ID No. 96)
5'-GAAGTAGGCTC*GCGCGG*CGAAAGGCACCAC-3' (SEQ ID No. 97)

Example 10

Introduction of the active site amino acid residues of the *A. niger* NRRL 3135 phytase into the consensus phytase-1

[0091] We used the crystal structure of the *Aspergillus niger* NRRL 3135 phytase to define all active site amino acid residues (see Reference Example and EP 897 010). Using the alignment of Figure 2, we replaced the following active site residues and additionally the non-identical adjacent ones of the consensus phytase -1 by those of the *A. niger* phytase:

S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S

[0092] The new protein sequence consensus phytase -7 was backtranslated into a DNA sequence (Figure 11) as described in Example 6. The corresponding gene (*fcp7*) was generated as described in Example 6 using the following oligonucleotide mixes:

Mix 1.7: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, CP-8.7, CP-9, CP-10.7
Mix 2.7: CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, CP-22.

[0093] The DNA sequences of the oligonucleotides are indicated in Figure 11. The newly synthesized oligonucleotides are additionally marked by number 7. After assembling of the oligonucleotides using the same PCR primers as mentioned in Example 6, the gene was cloned into an expression vector as described in Examples 11 - 13.

[0094] The pH-profile of consensus phytase-7, purified after expression in Hansenula polymorpha, was very similar to that of A. niger NRRL 3135 phytase (see Figure 19).

Example 11

Expression of the consensus phytase genes in *Hansenula polymorpha*

[0095] The phytase expression vectors, used to transform *H. polymorpha* RB11 (Gellissen *et al.*, 1994), were constructed by inserting the *Eco* RI fragment of pBsk⁻*fcp* or variants thereof into the multiple cloning site of the *H.*

polymorpha expression vector pFPMT121, which is based on an *ura3* selection marker from *S. cerevisiae*, a formate dehydrogenase (*FMD*) promoter element and a methanol oxidase (*MO*) termimator element from *H. polymorpha*. The 5' end of the *fcp* gene is fused to the *FMD* promoter, the 3' end to the *MOX* terminator (Gellissen *et al*., 1996; EP 0299 108 B). The resulting expression vectors were designated pFPMT*fcp*, pFPMT*fcp10*, pFPMT*fcp*7.

[0096] The constructed plasmids were propagated in *E. coli*. Plasmid DNA was purified using standard state of the art procedures. The expression plasmids were transformed into the *H. polymorpha* strain RP11 deficient in orotidine-5'-phosphate decarboxylase (*ura3*) using the procedure for preparation of competent cells and for transformation of yeast as described in Gelissen *et al*. (1996). Each transformation mixture was plated on YNB (0.14% w/v Difco YNB and 0.5% ammonium sulfate) containing 2% glucose and 1.8% agar and incubated at 37 °C. After 4 to 5 days individual transformant colonies were picked and grown in the liquid medium described above for 2 days at 37 °C. Subsequently, an aliquot of this culture was used to inoculate fresh vials with YNB-medium containing 2% glucose. After seven further passages in selective medium, the expression vector is integrated into the yeast genome in multimeric form. Subsequently, mitotically stable transformants were obtained by two additional cultivation steps in 3 ml non-selective liquid medium (YPD, 2% glucose, 10 g yeast extract, and 20 g peptone). In order to obtain genetically homogeneous recombinant strains an aliquot from the last stabilization culture was plated on a selective plate. Single colonies were isolated for analysis of phytase expression in YNB containing 2% glycerol instead of glucose to derepress the *fmd* promoter. Purification of the consensus phytases was done as described in Example 12.

Example 12

Expression of the consensus phytase genes in *Saccharomyces cerevisiae* and purification of the phytases from culture supernatant

[0097] The consensus phytase genes were isolated from the corresponding Bluescript-plasmid (pBsk$^-$*fcp*, pBSK$^-$*fcp10*, pBsk$^-$*fcp7*) and ligated into the *Eco* RI sites of the expression cassette of the *Saccharomyces cerevisiae* expression vector pYES2 (Invitrogen, San Diego, CA, USA) or subcloned between the shortened GAPFL (glyceraldhyde-3-phosphate dehydrogenase) promoter and the *pho5* terminator as described by Janes *et al*. (1990). The correct orientation of the gene was checked by PCR. Transformation of *S. cerevisiae* strains. e. g. INVSc1 (Invitrogen, San Diego, CA, USA) was done according to Hinnen *et al*. (1978). Single colonies harboring the phytase gene under the control of the GAPFL promoter were picked and cultivated in 5 ml selection medium (SD-uracil, Sherman *et al*., 1986) at 30°C under vigorous shaking (250 rpm) for one day. The preculture was then added to 500 ml YPD medium (Sherman *et al*., 1986) and grown under the same conditions. Induction of the *gal1* promoter was done according to the manufacturer's instructions. After four days of incubation cell broth was centrifuged (7000 rpm, GS3 rotor, 15 min, 5°C) to remove the cells and the supernatant was concentrated by way of ultrafiltration in Amicon 8400 cells (PM30 membranes) and ultrafree-15 centrifugal filter devices (Biomax-30K, Millipore, Bedford, MA, USA). The concentrate (10 ml) was desalted on a 40 ml Sephadex G25 Superfine column (Pharmacia Biotech, Freiburg, Germany), with 10 mM sodium acetate, pH 5.0, serving as elution buffer. The desalted sample was brought to 2 M $(NH_4)_2SO_4$ and directly loaded onto a 1 ml Butyl Sepharose 4 Fast Flow hydrophobic interaction chromatography column (Pharmacia Biotech, Freiburg, Germany) which was eluted with a linear gradient from 2 M to 0 M $(NH_4)_2SO_4$ in 10 mM sodium acetate, pH 5.0. Phytase was eluted in the break-through, concentrated and loaded on a 120 ml Sephacryl S-300 gel permeation chromatography column (Pharmacia Biotech, Freiburg, Germany). Consensus phytase -1 and consensus phytase -7 eluted as a homogeneous symmetrical peak and was shown by SDS-PAGE to be approx. 95% pure.

Example 13

Expression of the consensus phytase genes in *Aspergillus niger*

[0098] The Bluescript-plasmids pBsk$^-$*fcp*, pBSK$^-$*fcp10*, and pBsk$^-$*fcp7* were used as template for the introduction of a *Bsp* HI-site upstream of the start codon of the genes and an *Eco* RV-site downstream of the stop codon. The Expand™ High Fidelity PCR Kit (Boehringer Mannheim, Mannheim, Germany) was used with the following primers:

Primer Asp-1:

Bsp HI

5'-TATA*TCATGA*GCGTGTTCGTCGTGCTACTGTTC-3' (SEQ ID No. 98)

Primer Asp-2 used for cloning of *fcp* and *fcp7*:

Eco RV

3'-ACCCGACTTACAAAGCGAATT*CTATAG*ATATAT-5' (SEQ ID No. 99)

Primer Asp-3 used for cloning of *fcp10*:

Eco RV

3'-ACCCTTCTTACAAAGCGAATT*CTATAG*ATATAT-5' (SEQ ID No. 100)

[0099] The reaction was performed as described by the supplier. The PCR-amplified *fcp*-genes had a new *Bsp* HI site at the start codon, introduced by primer Asp-1, which resulted in a replacement of the second amino acid residue glycine by serine. Subsequently, the DNA-fragment was digested with *Bsp* HI and *Eco* RV and ligated into the *Nco* I site downstream of the glucoamylase promoter of *Aspergillus niger* (*glaA*) and the *Eco* RV site upstream of the *Aspergillus nidulans* tryptophan C terminator (*trpC*) (Mullaney *et al.*, 1985). After this cloning step, the genes were sequenced to detect possible failures introduced by PCR. The resulting expression plasmids which basically correspond to the pGLAC vector as described in Example 9 of EP 684 313 contained the orotidine-5'-phosphate decarboxylase gene (*pyr4*) of *Neurospora crassa* as a selection marker. Transformation of *Aspergillus niger* and expression of the consensus phytase genes was done as described in EP 684 313. The consensus phytases were purified as described in Example 12.

Example 14

Determination of phytase activity and of temperature optimum

[0100] Phytase activity was determined basically as described by Mitchell et al (1997). The activity was measured in an assay mixture containing 0.5% phytic acid (≈5 mM) in 200 mM sodium acetate, pH 5.0. After 15 min of incubation at 37 °C, the reaction was stopped by addition of an equal volume of 15% trichloroacetic acid. The liberated phosphate was quantified by mixing 100 µl of the assay mixture with 900 µl H_2O and 1 ml of 0.6 M H_2SO_4, 2% ascorbic acid and 0.5% ammonium molybdate. Standard solutions of potassium phosphate were used as reference. One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol phosphate per minute at 37 °C. The protein concentration was determined using the enzyme extinction coefficient at 280 nm calculated according to Pace et al (1995): consensus phytase -1.101; consensus phytase -7, 1.068; consensus phytase -1 10, 1.039.

[0101] In case of pH-optimum curves, purified enzymes were diluted in 10 mM sodium acetate, pH 5.0. Incubations were started by mixing aliquots of the diluted protein with an equal volume of 1% phytic acid (≈10 mM) in a series of different buffers: 0.4 M glycine/HCl, pH 2.5; 0.4 M acetate/NaOH, pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5; 0.4 M imidazole/HCl, pH 6.0, 6.5; 0.4 M Tris/HCl pH 7.0, 7.5, 8.0, 8.5, 9.0. Control experiments showed that pH was only slightly affected by the mixing step. Incubations were performed for 15 min at 37 °C as described above.

[0102] For determinations of the substrate specificities of the phytases, phytic acid in the assay mixture was replaced by 5 mM concentrations of the respective phosphate compounds. The activity tests were performed as described above.

[0103] For determination of the temperature optimum, enzyme (100 µl) and substrate solution (100 µl) were preincubated for 5 min at the given temperature. The reaction was started by addition of the substrate solution to the enzyme. After 15 min incubation, the reaction was stopped with trichloroacetic acid and the amount of phosphate released was determined.

[0104] The pH-optimum of the original consensus phytase was around pH 6.0-6.5 (80 U/mg). By introduction of the

Q50T mutation, the pH-optimum shifted to pH 6.0 (130 U/mg). After introduction of K91A, the pH optimum shifted one pH-unit into the acidic pH-range showing a higher specific activity between pH 2.5 and pH 6.0. That was shown for the stabilized mutants and for consensus phytase-10, too (Figure 15 and 16).

[0105] Consensus phytase-7, which was constructed to transfer the catalytic characteristics of the A. niger NRRL 3135 phytase into consensus phytase-1, had a pH-profile very similar to that of A. niger NRRL 3135 phytase (see Figure 19). The substrate specificity of consensus phytase-7 also resembled more to that of A. niger NRRL 3135 phytase than to that of consensus phytase-1.

[0106] The temperature optimum of consensus phytase-1 (71 °C) was 16-26 °C higher than the temperature optimum of the wild-type phytases (45-55 °C, Table 7) which were used to calculate the consensus sequence. The improved consensus phytase-10 showed a further increase of its temperature optimum to 80 °C (Figure 12). The temperature optimum of the consensus phytase-1-thermo[8] phytase was found in the same range (78 °C) when using the supernatant of an overproducing *S. cerevisiae* strain. The highest temperature optimum reached of 82 °C was determined for consensus phytase-10-thermo[3]-Q50T-K91A.

Table 7

Temperature optimum and T_m-value of consensus phytase and of the phytases from *A. fumigatus*, *A. niger*, *E. nidulans* and *M. thermophila*. The determination of the temperature optimum was performed as described in Example 14. The T_m-values were determined by differential scanning calorimetry as described in Example 15.

phytase	temperature optimum [°C]	Tm [°C]
Consensus phytase-10-thermo[3]-Q50T-K91A	82	89.3
Consensus phytase-10-thermo[3]-Q50T	82	88.6
Consensus phytase-10	80	85.4
Consensus phytase-1-thermo[8]-Q50T	78	84.7
Consensus phytase-1-thermo[8]-Q50T-K91A	78	85.7
Consensus phytase-1	71	78.1
A. niger NRRL3135	55	63.3
A. fumigatus 13073	55	62.5
A. fumigatus 13073 α-mutant	60	67.0
A. fumigatus 13073 α-mutant (optimized)	63	-
A. terreus 9A-1	49	57.5
A. terreus cbs.116.46	45	58.5
E. nidulans	45	55.7
M. thermophila	55	n. d.
T. thermophilus	45	n. d.

Example 15

Determination of the melting point by differential scanning calorimetry (DSC)

[0107] In order to determine the unfolding temperature of the phytases, differential scanning calorimetry was applied as previously published by Lehmann et al (2000). Solutions of 50-60 mg/ml homogeneous phytase were used for the tests. A constant heating rate of 10 °C/min was applied up to 90-95 °C.

[0108] The determined melting points reflect the results obtained for the temperature optima (Table 7). The most stable consensus phytase designed is consensus phytase-10-thermo[3]-Q50T-K91A showing a melting temperature

under the chosen conditions of 89.3 °C. This is 26 to 33.6 °C higher than the melting points of the wild-type phytases used.

Example 16

Transfer of basidiomycete phytase active site into consensus phytase-10-thermo[3]-Q50T-K91A

[0109] As described previously (Example 8), mutations derived from the basidiomycete phytase active site were introduced into the consensus phytase -10. The following five constructs a) to e) were prepared:

a) This construct is called consensus phytase -12, and it comprises a selected number of active site residues of the basidio consensus sequence. Its amino acid sequence (consphy12) is shown in Fig. 22 (the first 26 amino acids forms the signal peptide, amended positions are underlined);

b) a cluster of mutations (Cluster II) was transferred to the consensus phytase 10 sequence, viz.: S80Q, Y86F, S90G, K91A, S92A, K93T, A94R, Y95I;

c) analogously, another cluster of mutations (Cluster III) was transferred, viz.: T129V, E133A, Q143N, M136S, V137S, N138Q, S139A;

d) analogously, a further cluster of mutations (Cluster IV) was transferred, viz.: A168D, E171T, K172N, F173W;

e) and finally, a further cluster of mutations (Cluster V) was transferred, viz.: Q297G, S298D, G300D, Y305T.

[0110] These constructs were expressed as described in Examples 11 - 13.

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[0111]

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Annex to the application documents - subsequently filed sequence listing

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<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 93

cctgcactgg cgaatgcgtc aaccgggc 28

<210> 94
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 94

cacacgacaa caccatggtt tccatcttc 29

<210> 95
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 95

gaagatggaa accatggtgt tgtcgtgtg 29

<210> 96
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 96

gtggtgcctt tcgccgcgcg agcctacttc 30

<210> 97
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 97

gaagtaggct cgcgcggcga aaggcaccac 30

<210> 98
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 98

tatatcatga gcgtgttcgt cgtgctactg ttc 33

<210> 99
<211> 33
<212> DNA
<213> Artificial Sequence

SEQUENCE LISTING.txt

<220>
<223> Primer

<400> 99

acccgactta caaagcgaat tctatagata tat 33

<210> 100
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 100

accttctta caaagcgaat tctatagata tat 33

<210> 101
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus phytase-1

<400> 101

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Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser
  1               5                  10                  15
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp
            20                  25                  30
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp
        35                  40                  45
Gly Gln Tyr Ser Pro Tyr Phe Ser Leu Glu Asp Glu Ser Ala Ile Ser
    50                  55                  60
Pro Asp Val Pro Asp Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser
65                  70                  75                  80
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Ala Tyr Ser
                85                  90                  95
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys
            100                 105                 110
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu
        115                 120                 125
Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr
    130                 135                 140
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala
145                 150                 155                 160
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly
                165                 170                 175
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala
            180                 185                 190
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Ser Pro Val Ile Asp Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn
195 200 205
Thr Leu Asp His Gly Thr Cys Thr Ala Phe Glu Asp Ser Glu Leu Gly
210 215 220
Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg
225 230 235 240
Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp
245 250 255
Val Val Tyr Leu Met Asp Met Cys Pro Phe Glu Thr Val Ala Arg Thr
260 265 270
Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp
275 280 285
Glu Trp Arg Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly
290 295 300
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala
305 310 315 320
Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro Val Gln Asp His Thr
325 330 335
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn
340 345 350
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Ser Met Ile Ser Ile
355 360 365
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Ala Pro Leu Ser Thr Thr
370 375 380
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr
385 390 395 400
Val Pro Phe Gly Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala
405 410 415
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro
420 425 430
Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp
435 440 445
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu
450 455 460
Cys Phe Ala
465

<210> 102
<211> 1426
<212> DNA
<213> Artificial Sequence

<220>
<223> consensus phytase-1

<400> 102

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catccggtac cgccttgggt cctcgtggta attctcactc ttgtgacact gttgacggtg
120
gttaccaatg tttcccagaa atttctcact tgtggggtca atactctcca tacttctctt
180

SEQUENCE LISTING.txt

tggaagacga atctgctatt tctccagacg ttccagacga ctgtagagtt actttcgttc
240
aagttttgtc tagacacggt gctagatacc caacttcttc taagtctaag gcttactctg
300
ctttgattga agctattcaa aagaacgcta ctgctttcaa gggtaagtac gctttcttga
360
agacttacaa ctacactttg ggtgctgacg acttgactcc attcggtgaa aaccaaatgg
420
ttaactctgg tattaagttc tacagaagat acaaggcttt ggctagaaag attgttccat
480
tcattagagc ttctggttct gacagagtta ttgcttctgc tgaaaagttc attgaaggtt
540
tccaatctgc taagttggct gacccaggtt ctcaaccaca ccaagcttct ccagttattg
600
acgttattat tccagaagga tccggttaca acaacacttt ggaccacggt acttgtactg
660
ctttcgaaga ctctgaattg ggtgacgacg ttgaagctaa cttcactgct ttgttcgctc
720
cagctattag agctagattg gaagctgact tgccaggtgt tactttgact gacgaagacg
780
ttgtttactt gatggacatg tgtccattcg aaactgttgc tagaacttct gacgctactg
840
aattgtctcc attctgtgct ttgttcactc acgacgaatg gagacaatac gactacttgc
900
aatctttggg taagtactac ggttacggtg ctggtaaccc attgggtcca gctcaaggtg
960
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1080
ctgacttctc tcacgacaac tctatgattt ctattttctt cgctttgggt ttgtacaacg
1140
gtactgctcc attgtctact acttctgttg aatctattga agaaactgac ggttactctg
1200
cttcttggac tgttccattc ggtgctagag cttacgttga aatgatgcaa tgtcaagctg
1260
aaaaggaacc attggttaga gttttggtta acgacagagt tgttccattg cacggttgtg
1320
ctgttgacaa gttgggtaga tgtaagagag acgacttcgt tgaaggtttg tctttcgcta
1380
gatctggtgg taactgggct gaatgtttcg cttaagaatt catata
1426

<210> 103
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus phytase-10

<400> 103

SEQUENCE LISTING.txt

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Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp
            20                  25                  30
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp
        35                  40                  45
Gly Gln Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser
    50                  55                  60
Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser
65                  70                  75                  80
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Lys Tyr Ser
                85                  90                  95
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys
            100                 105                 110
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu
        115                 120                 125
Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile Lys Phe Tyr
    130                 135                 140
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Val Arg Ala
145                 150                 155                 160
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly
                165                 170                 175
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala
            180                 185                 190
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn
        195                 200                 205
Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Glu Leu Gly
    210                 215                 220
Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg
225                 230                 235                 240
Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr Asp Glu Asp
                245                 250                 255
Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr
            260                 265                 270
Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp
        275                 280                 285
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly
    290                 295                 300
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val
305                 310                 315                 320
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr
                325                 330                 335
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn
            340                 345                 350
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile
        355                 360                 365
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr
    370                 375                 380
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ala Ala Ser Trp Thr
385                 390                 395                 400
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala
                405                 410                 415
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro
            420                 425                 430
Leu His Gly Cys Gly Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp
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SEQUENCE LISTING.txt

435 440 445
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu
450 455 460
Cys Phe Ala
465

<210> 104
<211> 1426
<212> DNA
<213> Artificial Sequence

<220>
<223> consensus phytase-10

<400> 104

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120
gttaccaatg tttcccagaa atttctcact tgtggggtca atactctcca ttcttctctt
180
tggctgacga atctgctatt tctccagacg ttccaaaggg ttgtagagtt actttcgttc
240
aagttttgtc tagacacggt gctagatacc caacttcttc taagtctaag aagtactctg
300
ctttgattga agctattcaa aagaacgcta ctgctttcaa gggtaagtac gctttcttga
360
agacttacaa ctacactttg ggtgctgacg acttgactcc attcggtgaa caacaaatgg
420
ttaactctgg tattaagttc tacagaagat acaaggcttt ggctagaaag attgttccat
480
tcgttagagc ttctggttct gacagagtta ttgcttctgc tgaaaagttc attgaaggtt
540
tccaatctgc taagttggct gacccaggtg ctaacccaca ccaagcttct ccagttatta
600
acgttattat tccagaaggt gctggttaca acaacacttt ggaccacggt ttgtgtactg
660
ctttcgaaga atctgaattg ggtgacgacg ttgaagctaa cttcactgct gttttcgctc
720
cacctattag agctagattg gaagctcact tgccaggtgt taacttgact gacgaagacg
780
ttgttaactt gatggacatg tgtccattcg acactgttgc tagaacttct gacgctactc
840
aattgtctcc attctgtgac ttgttcactc acgacgaatg gattcaatac gactacttgc
900
aatctttggg taagtactac ggttacggtg ctggtaaccc attgggtcca gctcaaggtg
960
ttggtttcgt taacgaattg attgctagat tgactcactc tccagttcaa gaccacactt
1020
ctactaacca cactttggac tctaacccag ctactttccc attgaacgct actttgtacg
1080
ctgacttctc tcacgacaac actatggttt ctattttctt cgctttgggt ttgtacaacg

1140
gtactaagcc attgtctact acttctgttg aatctattga agaaactgac ggttacgctg
1200
cttcttggac tgttccattc gctgctagag cttacgttga aatgatgcaa tgtgaagctg
1260
aaaaggaacc attggttaga gttttggtta acgacagagt tgttccattg cacggttgtg
1320
gtgttgacaa gttgggtaga tgtaagagag acgacttcgt tgaaggtttg tctttcgcta
1380
gatctggtgg taactgggaa gaatgtttcg cttaagaatt catata
1426

<210> 105
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus phytase-1-thermo[8]-Q50T-K91A

<400> 105

Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser
1 5 10 15
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp
20 25 30
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp
35 40 45
Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser
50 55 60
Pro Asp Val Pro Asp Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser
65 70 75 80
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser
85 90 95
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys
100 105 110
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu
115 120 125
Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr
130 135 140
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala
145 150 155 160
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly
165 170 175
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala
180 185 190
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn
195 200 205
Thr Leu Asp His Gly Thr Cys Thr Ala Phe Glu Asp Ser Glu Leu Gly
210 215 220
Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg
225 230 235 240
Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp

245 250 255
Val Val Tyr Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr
260 265 270
Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp
275 280 285
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly
290 295 300
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala
305 310 315 320
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr
325 330 335
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn
340 345 350
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Ile Ser Ile
355 360 365
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr
370 375 380
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr
385 390 395 400
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala
405 410 415
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro
420 425 430
Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp
435 440 445
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu
450 455 460
Cys Phe Ala
465

<210> 106
<211> 1404
<212> DNA
<213> Artificial Sequence

<220>
<223> consensus phytase-1-thermo[8]-Q50T-K91A

<400> 106

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120
ttcccagaaa tttctcactt gtggggtacc tactctccat acttctcttt ggcagacgaa
180
tctgctattt ctccagacgt tccagacgac tgtagagtta ctttcgttca agttttgtct
240
agacacggtg ctagataccc aacttcttct gcgtctaagg cttactctgc tttgattgaa
300
gctattcaaa agaacgctac tgctttcaag ggtaagtacg ctttcttgaa gacttacaac
360
tacactttgg gtgctgacga cttgactcca ttcggtgaaa accaaatggt taactctggt

420
attaagttct acagaagata caaggctttg gctagaaaga ttgttccatt cattagagct
480
tctggttctg acagagttat tgcttctgct gaaaagttca ttgaaggttt ccaatctgct
540
aagttggctg acccaggttc tcaaccacac caagcttctc cagttattaa cgtgatcatt
600
ccagaaggat ccggttacaa caacactttg gaccacggta cttgtactgc tttcgaagac
660
tctgaattag gtgacgacgt tgaagctaac ttcactgctt tgttcgctcc agctattaga
720
gctagattgg aagctgactt gccaggtgtt actttgactg acgaagacgt tgtttacttg
780
atggacatgt gtccattcga cactgtcgct agaacttctg acgctactga attgtctcca
840
ttctgtgctt tgttcactca cgacgaatgg atccaatacg actacttgca aagcttgggt
900
aagtactacg gttacggtgc tggtaaccca ttgggtccag ctcaaggtgt tggtttcgct
960
aacgaattga ttgctagatt gactcactct ccagttcaag accacacttc tactaaccac
1020
actttggact ctaacccagc tactttccca ttgaacgcta ctttgtacgc tgacttctct
1080
cacgacaaca ctatgatatc tattttcttc gctttgggtt tgtacaacgg taccaagcca
1140
ttgtctacta cttctgttga atctattgaa gaaactgacg gttactctgc ttcttggact
1200
gttccattcg ctgctagagc ttacgttgaa atgatgcaat gtcaagctga aaaggaacca
1260
ttggttagag ttttggttaa cgacagagtt gttccattgc acggttgtgc tgttgacaag
1320
ttgggtagat gtaagagaga cgacttcgtt gaaggtttgt ctttcgctag atctggtggt
1380
aactgggctg aatgtttcgc ttaa
1404

<210> 107
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus phytase-10-thermo[3]-Q50T-K91A

<400> 107

Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser
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Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp
20 25 30
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp
35 40 45
Gly Thr Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser

SEQUENCE LISTING.txt

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     50                  55                  60
Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser
 65                  70                  75                  80
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser
                 85                  90                  95
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys
            100                 105                 110
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu
        115                 120                 125
Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile Lys Phe Tyr
    130                 135                 140
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala
145                 150                 155                 160
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly
                165                 170                 175
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala
            180                 185                 190
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn
        195                 200                 205
Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Glu Leu Gly
    210                 215                 220
Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg
225                 230                 235                 240
Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr Asp Glu Asp
                245                 250                 255
Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr
            260                 265                 270
Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp
        275                 280                 285
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly
    290                 295                 300
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val
305                 310                 315                 320
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr
                325                 330                 335
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn
            340                 345                 350
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile
        355                 360                 365
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr
    370                 375                 380
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr
385                 390                 395                 400
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala
                405                 410                 415
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro
            420                 425                 430
Leu His Gly Cys Gly Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp
        435                 440                 445
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu
    450                 455                 460
Cys Phe Ala
465
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SEQUENCE LISTING.txt

<210> 108
<211> 1404
<212> DNA
<213> Artificial Sequence

<220>
<223> consensus phytase-10-thermo-[3]-Q50T-K91A

<400> 108

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120
ttcccagaaa tttctcactt gtggggtaca tactctccat tcttctcttt ggctgacgaa
180
tctgctattt ctccagacgt tccaaagggt tgtagagtta ctttcgttca agttttgtct
240
agacacggtg ctagataccc aacttcttct gcgtctaagg cgtactctgc tttgattgaa
300
gctattcaaa agaacgctac tgctttcaag ggtaagtacg ctttcttgaa gacttacaac
360
tacactttgg gtgctgacga cttgactcca ttcggtgaac aacaaatggt taactctggt
420
attaagttct acagaagata caaggctttg gctagaaaga ttgttccatt cattagagct
480
tctggttctg acagagttat tgcttctgct gaaaagttca ttgaaggttt ccaatctgct
540
aagttggctg acccaggtgc taacccacac caagcttctc cagttattaa cgttattatt
600
ccagaaggtg ctggttacaa caacactttg gaccacggtt tgtgtactgc tttcgaagaa
660
tctgaattgg gtgacgacgt tgaagctaac ttcactgctg ttttcgctcc accaattaga
720
gctagattgg aagctcactt gccaggtgtt aacttgactg acgaagacgt tgttaacttg
780
atggacatgt gtccattcga cactgttgct agaacttctg acgctactca attgtctcca
840
ttctgtgact tgttcactca cgacgaatgg attcaatacg actacttgca atctttgggt
900
aagtactacg gttacggtgc tggtaaccca ttgggtccag ctcaaggtgt tggtttcgtt
960
aacgaattga ttgctagatt gactcactct ccagttcaag accacacttc tactaaccac
1020
actttggact ctaacccagc tactttccca ttgaacgcta ctttgtacgc tgacttctct
1080
cacgacaaca ctatggtttc tattttcttc gctttgggtt tgtacaacgg tactaagcca
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1200
gttccattcg ctgctagagc ttacgttgaa atgatgcaat gtgaagctga aaaggaacca
1260
ttggttagag ttttggttaa cgacagagtt gttccattgc acggttgtgg tgttgacaag
1320

SEQUENCE LISTING.txt

ttgggtagat gtaagagaga cgacttcgtt gaaggtttgt ctttcgctag atctggtggt
1380
aactgggaag aatgtttcgc ttaa
1404

<210> 109
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> A.fumigatus ATCC 13073 phytase alpha-mutant

<400> 109

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Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser
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Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn His Ser Lys Ser Cys
            20                  25                  30
Asp Thr Val Asp Leu Gly Tyr Gln Cys Ser Pro Ala Thr Ser His Leu
        35                  40                  45
Trp Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Glu Asp Glu Leu Ser Val
    50                  55                  60
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Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Gln
65                  70                  75                  80

Arg His Gly Ala Arg Phe Pro Thr Ser Gly Ala Ala Thr Arg Ile Ser
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Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys
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Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu
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Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala
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Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn
        195                 200                 205

Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Glu Leu Gly
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Leu His Gly Cys Gly Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp
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Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu
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Cys Phe Ala
465
```

Claims

1. A fermentation assembly comprising

   a vessel suitable for carrying out reactions involving living cells;

   at least two storage flasks connected to said vessel for supply of liquids and means to transport said liquids from said storage flasks to said vessel;

   individual appliances monitoring the supply of the contents of said storage flasks to said vessel;

a harvest flask connected to said vessel and means to transport fermentation broth from said vessel to said harvest flask; and

a device for controlling and maintaining a constant dilution rate in said vessel with varying rates of individual supply of liquid from said storage flasks to said vessel.

2. An assembly as in claim 1 and in accordance with Figure 1 comprising

a fermentor 1 equipped with inlet tubes 2a from storage flasks 2 for supply of liquids; pumps 3 for transporting liquids from the storage flasks 2 to fermentor 1; scales 4 for monitoring the amount of liquids supplies to and discharged from the fermentor; gas inlet tubes 9 and outlet tubes 10; pump 6 for discharging fermentation broth via outlet tubes 5a to a harvest flask 5; main controlling unit 7 for overall process monitoring and steering; controlling unit 11 for monitoring and steering individual control systems 17 for temperature, pH, gas pressure, fermentor content and antifoam agents; circuit 12 including pump 13 for gas supply and taking samples; gas inlet and outlet flow control 14 and 15; and, optionally, sterile filters 16 and thermostating unit 8.

3. An assembly as in claims 1 or 2, wherein said storage flasks comprise individual flasks for solutions of carbon, nitrogen, and mineral sources required for the growth of said cells and optimal formation of the desired reaction product.

4. An assembly as in any one of claims 1 to 3, wherein said storage flasks comprise at least one individual flask containing a controlling agent.

5. An assembly as in any one of claims 1 to 4, wherein said storage flasks comprise an individual flask containing water.

6. An assembly as in any one of claims 1 to 5, wherein said vessel contains a fixed bed and/or an expanded bed and/or a moving bed of immobilized living cells.

7. An assembly as in claim 6 wherein the living cells are immobilized on a porous carrier.

8. A continuous process for the manufacture of proteins from cultures of living cells in which process the nutrients and other agents required for the growth of the cells and the optimal production of the desired protein are fed into the reactor individually at a constant dilution rate.

9. A continuous process according to claim 8 wherein the protein is selected from the group consisting of catalase, lactase, phenoloxidase, oxidase, oxidoreductase, glucanase cellulase, xylanase and other polysaccharide, peroxidase, lipase, hydrolase, esterase, cutinase, protease and other proteolytic enzymes, aminopeptidase, carboxypeptidase, phytase, lyase, pectinase and other pectinolytic enzymes, amylase, glucosidase, mannosidase, isomerase, invertase, transferase, ribonuclease, chitinase, and desoxyribonuclease or the protein is selected from the group of therapeutic proteins such as antibodies, vaccines, antigens, or of antibacterial and/or health-beneficial proteins such as lactoternin, lactoperoxidase or lysozyme.

10. A continuous process according to claim 8 wherein the protein is selected from the group consisting of proteins having the activity of a therapeutic protein such as antibodies, vaccines, antigens.

11. A process as in any one of claims 8 to 10 wherein the cells are immobilized.

12. A process as in any one of claims 8 to 11 wherein the cell is a phytase-producing microorganism.

13. A process as in claim 12, wherein the phytase-producing microorganism is *Hansenula polymorpha*.

14. A process as in claim 13, wherein the phytase-producing microorganism is *Hansenula polymorpha* transformed by a DNA encoding a phytase of fungal or consensus origin.

15. A process as in any one of claims 8 to 14, wherein the cell or microorganism is in a fixed bed and/or an expanded bed and/or a moving bed on a porous carrier.

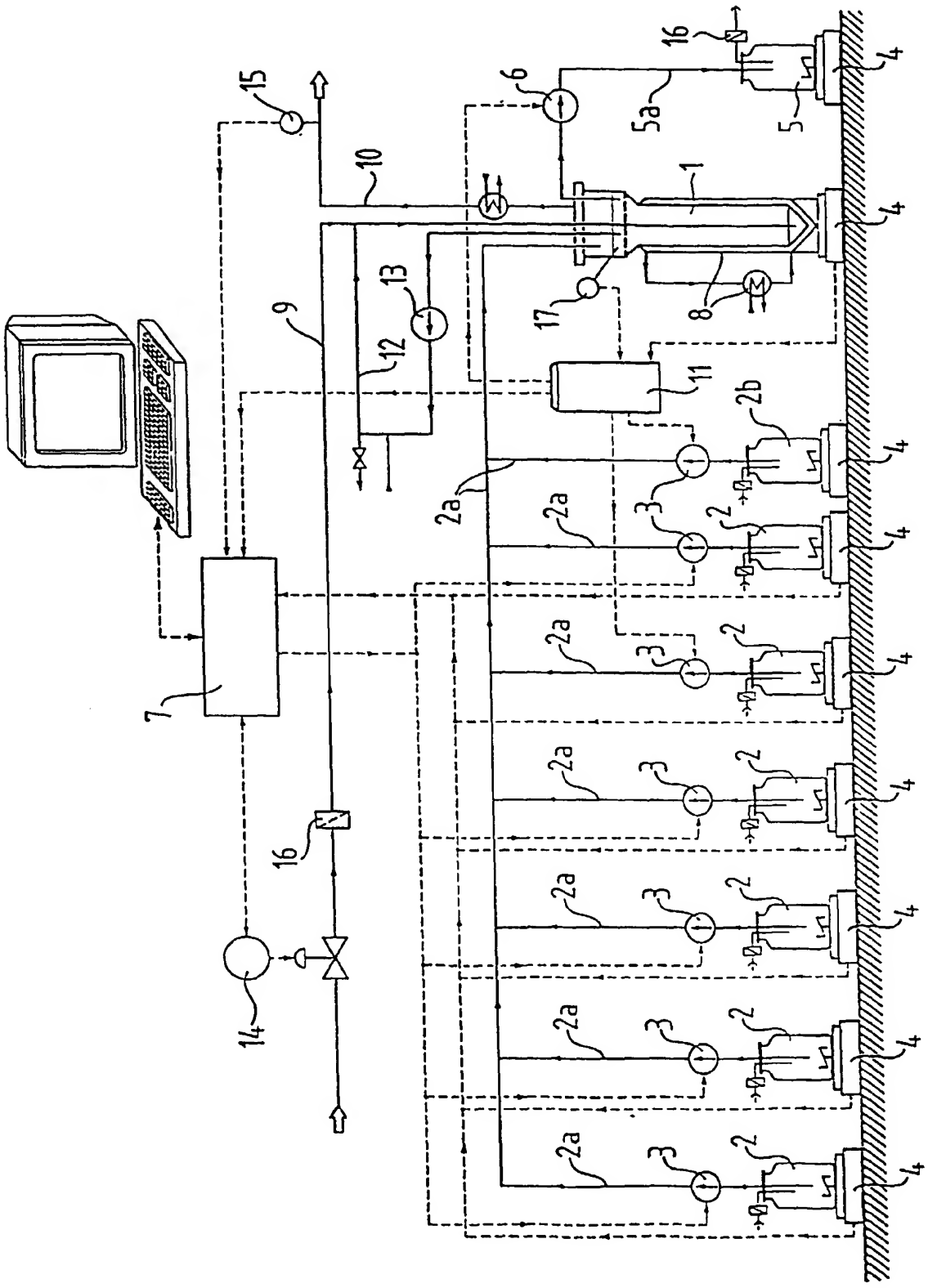
16. A process as in any one of claims 8 to 15, wherein the carbon source is glycerol or a sugar like a mono-, di- or polysaccharide.

17. A process as in claim 16, wherein the carbon source is glucose.

18. A process as in any one of claims 8 to 15, wherein the carbon source is methanol.

19. A process as in any one of claims 8 to 15, wherein the carbon source is glucose and methanol.

20. A process as in 19, wherein the total amount of methanol and glucose is from about 10 to about 500 g/l each.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17

Figure 2

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A. niger var. awamori     NqsTCDTVDQ GYQCFSETSH LWGQYAPFFS
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A. fumigatus 32239        GSkACDTVEl GYQCsPGTSH LWGQYSPFFS
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NNTLDHSVCT NFEA...SEL
E. nidulans              FRKAQLhDHG S..gQATPVV NVIIPEiDGF
NNTLDHSTCV SFEN...DEr
T. thermophilus          FQSAKVlDPh SDkHDAPPTI NVIIeEGPSY
NNTLDtGSCP VFED...SSg
M. thermophila           FHSAlLADRG STvRPTlPyd mVVIPETAGa
NNTLHNDlCT AFEEgpySTI

Consensus                FQSAKLADPG S-PHQASPVI NVIIPEGSGY
NNTLDHGTCT AFED---SEL
Consensus phytase        FQSAKLADPG SQPHQASPVI DVIIPEGSGY
NNTLDHGTCT AFED...SEL

                                201
250
A. terreus 9A-1          GDDAvANFTA VFAPAIaQRL EADLPGVqLS
TDDVVnLMAM CPFETVSlTD
A. terreus cbs           GDAAADNFTA VFAPAIakRL EADLPGVqLS
ADDVVnLMAM CPFETVSlTD
A. niger var. awamori    ADTVEANFTA TFAPSIRQRL ENDLSGVTLT
DTEVTyLMDM CSFDTIStST
A. niger T213            ADTVEANFTA TFAPSIRQRL ENDLSGVTLT
DTEVTyLMDM CSFDTIStST
A. niger NRRL3135        ADTVEANFTA TFVPSIRQRL ENDLSGVTLT
DTEVTyLMDM CSFDTIStST
A. fumigatus 13073       GDEVAANFTA lFAPDIRARa EkHLPGVTLT
DEDVVsLMDM CSFDTVARTS
A. fumigatus 32722       GDEVAANFTA lFAPDIRARa EkHLPGVTLT
DEDVVsLMDM CSFDTVARTS
A. fumigatus 58128       GDEVAANFTA lFAPDIRARa EkHLPGVTLT
DEDVVsLMDM CSFDTVARTS
A. fumigatus 26906       GDEVAANFTA lFAPDIRARa KkHLPGVTLT
DEDVVsLMDM CSFDTVARTS
A. fumigatus 32239       GDEVEANFTA lFAPAIRARI EkHLPGVqLT
DDDVVsLMDM CSFDTVARTA
E. nidulans              ADEiEANFTA IMGPPIRkRL ENDLPGIKLT
NENVIyLMDM CSFDTMARTA
T. thermophilus          GHDAQEKFAk qFAPAIlEKI KDHLPGVDLA
vSDVpyLMDL CPFETLARNh
M. thermophila           GDDAQDTYlS TFAGPItARV NANLPGANLT
DADTVaLMDL CPFETVAsSS
```

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Consensus                GDDAEANFTA TFAPAIRARL EADLPGVTLT DEDVV-
LMDM CPFETVARTS
Consensus phytase        GDDVEANFTA LFAPAIRARL EADLPGVTLT
DEDVVYLMDM CPFETVARTS


                         251
300
A. terreus 9A-1          ........ ...DAhTLSPFC DLFTAtEWtq
YNYLlSLDKY YGYGGGNPLG
A. terreus cbs           ........ ...DAhTLSPFC DLFTAaEWtq
YNYLlSLDKY YGYGGGNPLG
A. niger var. awamori    ........ ...vDTKLSPFC DLFTHdEWih
YDYLQSLkKY YGHGAGNPLG
A. niger T213            ........ ...vDTKLSPFC DLFTHdEWih
YDYLRSLkKY YGHGAGNPLG
A. niger NRRL3135        ........ ...vDTKLSPFC DLFTHdEWin
YDYLQSLkKY YGHGAGNPLG
A. fumigatus 13073       ........ ...DASQLSPFC QLFTHnEWkk
YNYLQSLGKY YGYGAGNPLG
A. fumigatus 32722       ........ ...DASQLSPFC QLFTHnEWkk
YNYLQSLGKY YGYGAGNPLG
A. fumigatus 58128       ........ ...DASQLSPFC QLFTHnEWkk
YNYLQSLGKY YGYGAGNPLG
A. fumigatus 26906       ........ ...DASQLSPFC QLFTHnEWkk
YNYLQSLGKY YGYGAGNPLG
A. fumigatus 32239       ........ ...DASELSPFC AIFTHnEWkk
YDYLQSLGKY YGYGAGNPLG
E. nidulans              ........ ...HGTELSPFC AIFTEkEWlq
YDYLQSLSKY YGYGAGSPLG
T. thermophilus          ........ ...TDT.LSPFC ALsTQeEWqa
YDYYQSLGKY YGnGGGNPLG
M. thermophila           sdpatadagg gNGrpLSPFC rLFSEsEWra
YDYLQSVGKW YGYGPGNPLG

Consensus                ---------- -DATELSPFC ALFTE-EW--
YDYLQSLGKY YGYGAGNPLG
Consensus phytase        .......... .DATELSPFC ALFTHDEWRQ
YDYLQSLGKY YGYGAGNPLG
```

```
                                301
350
A. terreus 9A-1          PVQGVGWaNE LMARLTRAPV HDHTCVNNTL
DASPATFPLN ATLYADFSHD
A. terreus cbs           PVQGVGWaNE LIARLTRSPV HDHTCVNNTL
DANPATFPLN ATLYADFSHD
A. niger var. awamori    PTQGVGYaNE LIARLTHSPV HDDTSSNHTL
DSNPATFPLN STLYADFSHD
A. niger T213            PTQGVGYaNE LIARLTHSPV HDDTSSNHTL
DSNPATFPLN STLYADFSHD
A. niger NRRL3135        PTQGVGYaNE LIARLTHSPV HDDTSSNHTL
DSSPATFPLN STLYADFSHD
A. fumigatus 13073       PAQGIGFtNE LIARLTRSPV QDHTSTNsTL
vSNPATFPLN ATMYVDFSHD
A. fumigatus 32722       PAQGIGFtNE LIARLTRSPV QDHTSTNsTL
vSNPATFPLN ATMYVDFSHD
A. fumigatus 58128       PAQGIGFtNE LIARLTRSPV QDHTSTNsTL
vSNPATFPLN ATMYVDFSHD
A. fumigatus 26906       PAQGIGFtNE LIARLTRSPV QDHTSTNsTL
vSNPATFPLN ATMYVDFSHD
A. fumigatus 32239       PAQGIGFtNE LIARLTNSPV QDHTSTNsTL
DSDPATFPLN ATIYVDFSHD
E. nidulans              PAQGIGFtNE LIARLTQSPV QDNTSTNHTL
DSNPATFPLD rKLYADFSHD
T. thermophilus          PAQGVGFvNE LIARMTHSPV QDYTTVNHTL
DSNPATFPLN ATLYADFSHD
M. thermophila           PTQGVGFvNE LLARLAgvPV RDgTSTNRTL
DGDPrTFPLG rPLYADFSHD

Consensus                PAQGVGF-NE LIARLTHSPV QDHTSTNHTL
DSNPATFPLN ATLYADFSHD
Consensus phytase        PAQGVGFANE LIARLTRSPV QDHTSTNHTL
DSNPATFPLN ATLYADFSHD

                                351
400
A. terreus 9A-1          SNLVSIFWAL GLYNGTAPLS qTSVESVSQT
DGYAAAWTVP FAARAYVEMM
A. terreus cbs           SNLVSIFWAL GLYNGTkPLS qTTVEDITrT
DGYAAAWTVP FAARAYIEMM
A. niger var. awamori    NGIISILFAL GLYNGTkPLS TTTVENITQT
DGFSSAWTVP FASRlYVEMM
A. niger T213            NGIISILFAL GLYNGTkPLS TTTVENITQT
DGFSSAWTVP FASRlYVEMM
A. niger NRRL3135        NGIISILFAL GLYNGTkPLS TTTVENITQT
DGFSSAWTVP FASRlYVEMM
A. fumigatus 13073       NSMVSIFFAL GLYNGTEPLS rTSVESaKEl
DGYSASWVVP FGARAYFEtM
A. fumigatus 32722       NSMVSIFFAL GLYNGTGPLS rTSVESaKEl
DGYSASWVVP FGARAYFEtM
A. fumigatus 58128       NSMVSIFFAL GLYNGTEPLS rTSVESaKEl
DGYSASWVVP FGARAYFEtM
A. fumigatus 26906       NSMVSIFFAL GLYNGTEPLS rTSVESaKEl
DGYSASWVVP FGARAYFEtM
A. fumigatus 32239       NGMIPIFFAM GLYNGTEPLS qTSeESTKES
NGYSASWAVP FGARAYFEtM
E. nidulans              NSMISIFFAM GLYNGTQPLS mDSVESIQEm
DGYAASWTVP FGARAYFELM
T. thermophilus          NTMTSIFaAL GLYNGTAkLS TTEIKSIEET
DGYSAAWTVP FGGRAYIEMM
M. thermophila           NDMMGVLgAL GaYDGVPPLD KTArrDpEEl
GGYAASWAVP FAARiYVEKM
```

```
Consensus                NSMISIFFAL GLYNGTAPLS TTSVESIEET
DGYAASWTVP FGARAYVEMM
Consensus phytase        NSMISIFFAL GLYNGTAPLS TTSVESIEET
DGYSASWTVP FGARAYVEMM

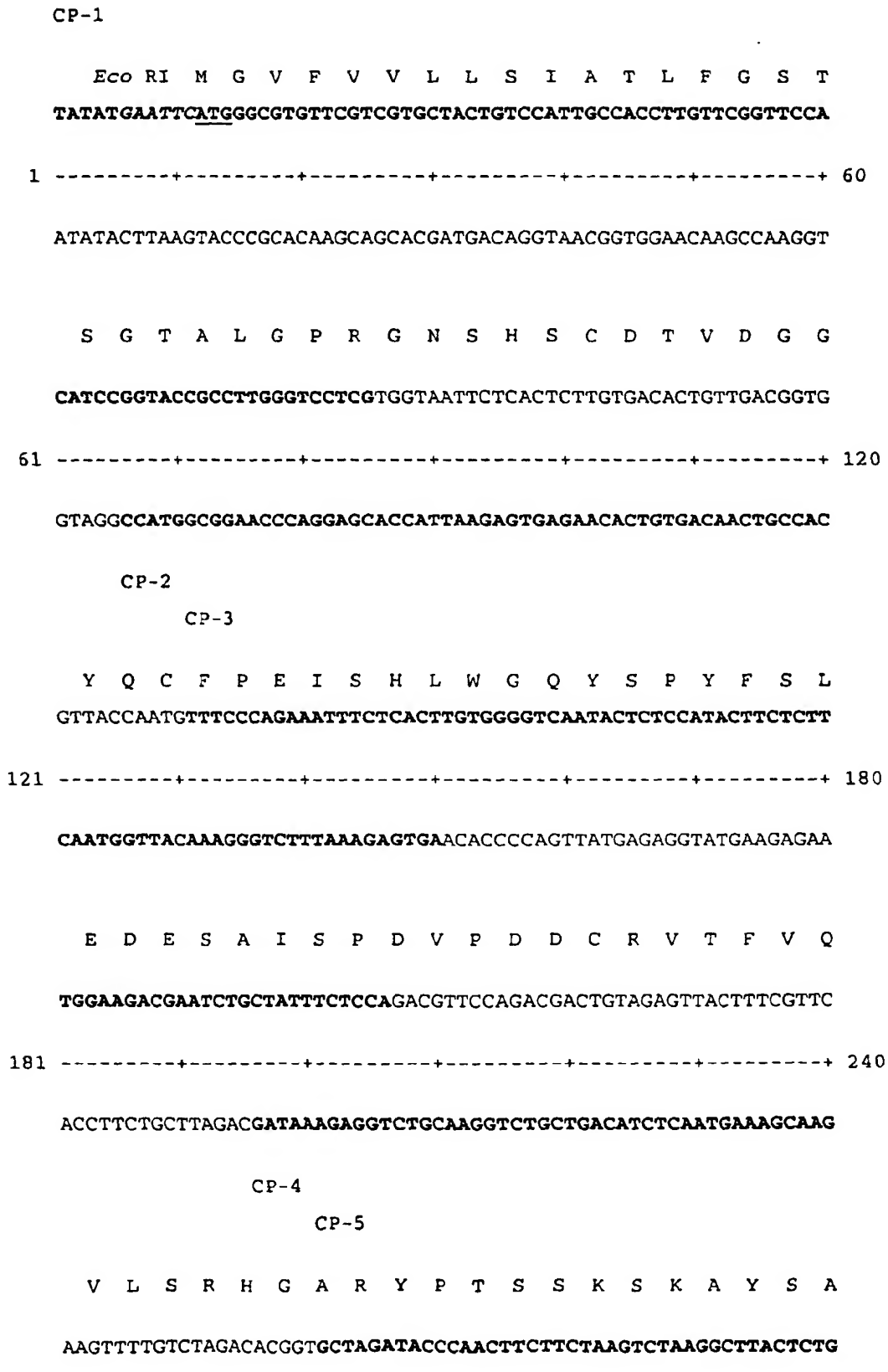

                                401
450
A. terreus 9A-1          QC........ .....RAEKE PLVRVLVNDR
VMPLHGCPTD KLGRCKrDAF
A. terreus cbs           QC........ .....RAEKQ PLVRVLVNDR
VMPLHGCAVD NLGRCKrDDF
A. niger var. awamori    QC........ .....QAEQE PLVRVLVNDR
VVPLHGCPID aLGRCTrDSF
A. niger T213            QC........ .....QAEQE PLVRVLVNDR
VVPLHGCPID aLGRCTrDSF
A. niger NRRL3135        QC........ .....QAEQE PLVRVLVNDR
VVPLHGCPVD aLGRCTrDSF
A. fumigatus 13073       QC........ .....KSEKE PLVRALINDR
VVPLHGCDVD KLGRCKLNDF
A. fumigatus 32722       QC........ .....KSEKE PLVRALINDR
VVPLHGCDVD KLGRCKLNDF
A. fumigatus 58128       QC........ .....KSEKE SLVRALINDR
VVPLHGCDVD KLGRCKLNDF
A. fumigatus 26906       QC........ .....KSEKE PLVRALINDR
VVPLHGCDVD KLGRCKLNDF
A. fumigatus 32239       QC........ .....KSEKE PLVRALINDR
VVPLHGCAVD KLGRCKLKDF
E. nidulans              QC........ .....E.KKE PLVRVLVNDR
VVPLHGCAVD KFGRCTLDDW
T. thermophilus          QC........ .....DDSDE PVVRVLVNDR
VVPLHGCEVD SLGRCKrDDF
M. thermophila           RCsgggggggg ggegrQEKDE eMVRVLVNDR
VMTLkGCGAD ErGMCTLErF

Consensus                QC-------- -----QAEKE PLVRVLVNDR
VVPLHGCAVD KLGRCKLDDF
Consensus phytase        QC........ .....QAEKE PLVRVLVNDR
VVPLHGCAVD KLGRCKRDDF


                                     451
471
A. terreus 9A-1          VAGLSFAQAG GNWADCF~~~ ~
A. terreus cbs           VEGLSFARAG
GNWAECF~~~ ~
A. niger var. awamori    VrGLSFARSG GDWAECsA~~ ~
A. niger T213            VrGLSFARSG GDWAECFA~~ ~
A. niger NRRL3135        VrGLSFARSG
GDWAECFA~~ ~
A. fumigatus 13073       VKGLSWARSG GNWGECFS~~ ~
A. fumigatus 32722       VKGLSWARSG GNWGECFS~~ ~
A. fumigatus 58128       VKGLSWARSG GNWGECFS~~ ~
A. fumigatus 26906       VKGLSWARSG GNWGECFS~~ ~
A. fumigatus 32239       VKGLSWARSG
GNSEQSFS~~ ~
E. nidulans              VEGLNFARSG GNWkTCFTl~ ~
T. thermophilus          VrGLSFARqG GNWEGCYAas e
M. thermophila           IESMAFARGN GKWDlCFA~~ ~

Consensus                VEGLSFARSG GNWAECFA-- -
Consensus phytase        VEGLSFARSG GNWAECFA.. .
```

Figure 3


CP-1
Eco RI M G V F V V L L S I A T L F G S T
TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
1 ---------+---------+---------+---------+---------+---------+ 60
ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT
S G T A L G P R G N S H S C D T V D G G
CATCCGGTACCGCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG
61 ---------+---------+---------+---------+---------+---------+ 120
GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC
CP-2
CP-3
Y Q C F P E I S H L W G Q Y S P Y F S L
GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
121 ---------+---------+---------+---------+---------+---------+ 180
CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA
E D E S A I S P D V P D D C R V T F V Q
TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTC
181 ---------+---------+---------+---------+---------+---------+ 240
ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG
CP-4
CP-5
V L S R H G A R Y P T S S K S K A Y S A
AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGGCTTACTCTG

241 ---------+---------+---------+---------+---------+---------+ 300

TTCAAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTCAGATTCCGAATGAGAC

L I E A I Q K N A T A F K G K Y A F L K

CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA

301 ---------+---------+---------+---------+---------+---------+ 360

GAAACTAACTTCGATAAGTTTTCTT**GCGATGACGAAAGTTCCCATTCATGCGAAAGAACT**

CP-6

CP-7

T Y N Y T L G A D D L T P F G E N Q M V

AGACTTACAACTACACTTTGGGTGCTGACG**ACTTGACTCCATTCGGTGAAAACCAAATGG**

361 ---------+---------+---------+---------+---------+---------+ 420

TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

N S G I K F Y R R Y K A L A R K I V P F

TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT

421 ---------+---------+---------+---------+---------+---------+ 480

AATTGAGACCATAATTCAAGATGTCTTCTATGTTC**CGAAACCGATCTTTCTAACAAGGTA**

CP-8

CP-9

I R A S G S D R V I A S A E K F I E G F

TCATTAGAGCTTCTGGTTCTGACAGAGTTATTGCTTCTGC**TGAAAAGTTCATTGAAGGTT**

481 ---------+---------+---------+---------+---------+---------+ 540

AGTAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

```
     Q  S  A  K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  D
    TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG

541 ---------+---------+---------+---------+---------+---------+ 600

    AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAAC

                                                 CP-10
                                                      CP-11

     V  I  I  P  E  G  S  G  Y  N  N  T  L  D  H  G  T  C  T  A
    ACGTTATTATTCCAGAAGGaTCcGGTTACAACAACACTTTGGACCACGGTACTTGTACTG

601 ---------+---------+---------+---------+---------+---------+ 660

    TGCAATAATAAGGTCTTCCtAGgCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGAC


     F  E  D  S  E  L  G  D  D  V  E  A  N  F  T  A  L  F  A  P

    CTTTCGAAGACTCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTCGCTC

661 ---------+---------+---------+---------+---------+---------+ 720

    GAAAGCTTCTGAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAG

                                                          CP-12

     A  I  R  A  R  L  E  A  D  L  P  G  V  T  L  T  D  E  D  V

    CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACG

721 ---------+---------+---------+---------+---------+---------+ 780

    GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGC


    CP-13

     V  Y  L  M  D  M  C  P  F  E  T  V  A  R  T  S  D  A  T  E

    TTGTTTACTTGATGGACATGTGTCCATTCGAAACTGTTGCTAGAACTTCTGACGCTACTG

781 ---------+---------+---------+---------+---------+---------+ 840
```

AACAAATGAACTACCTGTACACAGGTAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

L S P F C A L F T H D E W R Q Y D Y L Q

AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGACAATACGACTACTTGC

841 ---------+---------+---------+---------+---------+---------+ 900

TTAAC**AGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTGTTATGCTGATGAACG**

CP-14

CP-15

S L G K Y Y G Y G A G N P L G P A Q G V

AATCTTTGGG**TAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG**

901 ---------+---------+---------+---------+---------+---------+ 960

TTAGAAACCCATTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCAC

G F A N E L I A R L T R S P V Q D H T S

TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT

961 ---------+---------+---------+---------+---------+---------+
1020

AACCAAAGCGATTGC**TTAACTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA**

CP-16

CP-17

T N H T L D S N P A T F P L N A T L Y A

CTACTAACCACACTTTGGAC**TCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG**

1021 ---------+---------+---------+---------+---------+---------+
1080

GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

```
          D  F  S  H  D  N  S  M  I  S  I  F  F  A  L  G  L  Y  N  G

        CTGACTTCTCTCACGACAACTCTATGATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG

    1081 ---------+---------+---------+---------+---------+---------+
1140

        GACTGAAGAGAGTGCTGTTGAGATACTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC

                                  CP-18
                                       CP-19

          T  A  P  L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A

        GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTG

    1141 ---------+---------+---------+---------+---------+---------+
1200

        CATGACGAGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGAC


          S  W  T  V  P  F  G  A  R  A  Y  V  E  M  M  Q  C  Q  A  E

        CTTCTTGGACTGTTCCATTCGGTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
    1201 ---------+---------+---------+---------+---------+---------+
1260

        GAAGAACCTGACAAGGTAAGCCACGATCTCGAATGCAACTTTACTACGTTACAGTTCGAC

                                            CP-20
                                                 CP-21

          K  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A

        AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG

    1261 ---------+---------+---------+---------+---------+---------+
1320

        TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC
```

```
          V  D  K  L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R

          CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA

    1321 ---------+---------+---------+---------+---------+---------+
1380

          GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT

                                                          CP-22
            S  G  G  N  W  A  E  C  F  A  *  Eco RI

          GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA

    1381 ---------+---------+---------+---------+------ 1426

          CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT
```

Figure 4

```
                      1
50

P. involutus (phyA1) SvP.KnTAPt FPIPeseQrn WSPYSPYFPL AeYkAPPAGC
QInQVNIIQR

P. involutus (phyA2) SvP.RniAPK FSIPeseQrn WSPYSPYFPL AeYkAPPAGC
EInQVNIIQR

T. pubescens         hiPlRdTSAc LdVTrDvQqs WSmYSPYFPa AtYvAPPASC
QInQVHIIQR

A. pediades          GgvvQaTfvQ pfFPpQiQds WAAYTPYYPV qaYtPPPkDC
KItQVNIIQR

P. lycii             StQfsfvAAQ LPIPaQntsn WGPYdPFFPV EpYaAPPEGC
tVtQVNLIQR
```

Basidio S-P-R-TAAQ LPIP-Q-Q-- WSPYSPYFPV A-Y-APPAGC QI-QVNIIQR

51

100

P. involutus (*phyA1*) HGARFPTSGA TTRIKAGLTK LQGvqnfTDA KFNFIkSfkY dLGnsDLVPF

P. involutus (*phyA2*) HGARFPTSGA ATRIKAGLSK LQSvqnfTDP KFDFIkSfTY dLGtsDLVPF

T. pubescens HGARFPTSGA AkRIQTAVAK LKAAsnyTDP lLAFVtNyTY sLGqDsLVeL

A. pediades HGARFPTSGA GTRIQAAVkK LQSAktyTDP RLDFLtNyTY tLGhDDLVPF

P. lycii HGARWPTSGA rSRqvAAVAK IQmArpfTDP KYEFLnDfvY kFGvADLLPF

Basidio HGARFPTSGA ATRIQAAVAK LQSA---TDP KLDFL-N-TY -LG-DDLVPF

101

150

P. involutus (*phyA1*) GAaQSfDAGQ EAFARYSkLV SkNNLPFIRA dGSDRVVDSA TNWTAGFAsA

P. involutus (*phyA2*) GAaQSfDAGl EvFARYSkLV SsDNLPFIRS dGSDRVVDTA TNWTAGFAsA

T. pubescens GAtQSSEAGQ EAFTRYSsLV SaDELPFVRA SGSDRVVATA nNWTAGFAlA

A. pediades GAlQSSQAGE ETFqRYSfLV SkENLPFVRA SSSNRVVDSA TNWTEGFSaA

P. lycii GAnQShQTGt DmYTRYStLf egGDVPFVRA AGdQRVVDSS TNWTAGFGdA

Basidio GA-QSSQAGQ EAFTRYS-LV S-DNLPFVRA SGSDRVVDSA TNWTAGFA-A

151

200

P. involutus (*phyA1*) ShNTvqPkLn LILPQtGNDT LEDNMCPaAG DSDPQvNaWL AVafPSITAR

P. involutus (*phyA2*) SrNAiqPkLd LILPQtGNDT LEDNMCPaAG ESDPQvDaWL AsafPSVTAQ

T. pubescens SsNSitPvLs VIISEaGNDT LDDNMCPaAG DSDPQvNqWL AqFAPPMTAR

A. pediades ShHvlnPiLf VILSEslNDT LDDaMCPnAG sSDPQtGiWt SIYGTPIAnR

P. lycii SgETvlPtLq VVLqEeGNcT LcNNMCPnEv DGDest.tWL GVFAPnITAR

Basidio S-NT--P-L- VILSE-GNDT LDDNMCP-AG DSDPQ-N-WL AVFAPPITAR

201

250

P. involutus (*phyA1*) LNAAAPSvNL TDtDAfNLvs LCAFlTVSkE kkSdFCtLFE giPGsFeAFa

P. involutus (phyA2) LNAAAPGANL TDaDAfNLvs LCPFmTVSkE qkSdFCtLFE giPGsFeAFa

T. pubescens LNAGAPGANL TDtDTyNL1t LCPFETVAtE rrSeFCDIYE elQAE.dAFa

A. pediades LNqqAPGANI TAaDvsNLip LCAFETIvkE tpSpFCNLF. .tPEEFaqFe

P. lycii LNAAAPSANL SDsDAltLmd MCPFDTLSsG naSpFCDLF. .tAEEYvSYe

Basidio **LNAAAPGANL TD-DA-NL-- LCPFETVS-E --S-FCDLFE --PEEF-AF-**

251

300

P. involutus (phyA1) YgGDLDKFYG TGYGQeLGPV QGVGYVNELI ARLTnsAVRD NTQTNRTLDA

P. involutus (phyA2) YaGDLDKFYG TGYGQALGPV QGVGYINELL ARLTnsAVnD NTQTNRTLDA

T. pubescens YnADLDKFYG TGYGQPLGPV QGVGYINELI ARLTaQnVsD HTQTNsTLDS

A. pediades YfGDLDKFYG TGYGQPLGPV QGVGYINELL ARLTemPVRD NTQTNRTLDS

P. lycii YyyDLDKYYG TGpGNALGPV QGVGYVNELL ARLTgQAVRD ETQTNRTLDS

Basidio **Y-GDLDKFYG TGYGQPLGPV QGVGYINELL ARLT-QAVRD NTQTNRTLDS**

301

350

P. involutus (phyA1) SPvTFPLNKT FYADFSHDNl MVAVFSAMGL FrQPAPLsTS vPNPwRTWrT

P. involutus (phyA2) APdTFPLNKT MYADFSHDNl MVAVFSAMGL FrQSAPLsTS tPDPNRTWLT

T. pubescens SPeTFPLNRT LYADFSHDNQ MVAIFSAMGL FNQSAPLDPT tPDPaRTFLv

A. pediades SPlTFPLDRS IYADLSHDNQ MIAIFSAMGL FNQSSPLDPS fPNPKRTWVT

P. lycii dPaTFPLNRT FYADFSHDNt MVPIFAALGL FNaTA.LDPl kPDeNRlWVd

Basidio **SP-TFPLNRT FYADFSHDNQ MVAIFSAMGL FNQSAPLDPS - PDPNRTWVT**

351

400

P. involutus (phyA1) SsLVPFSGRM VVERLsC..f GT..... .....tkV RVLVQDqVQP

P. involutus (phyA2) SsVVPFSARM aVERLsC..a GT..... .....tkV RVLVQDqVQP

T. pubescens kKIVPFSARM VVERLdC..g GA..... .....qsV RLLVNDAVQP

A. pediades SRLtPFSARM VtERLlCqrd GTgsggpsri mrngnvqtfV RILVNDALQP

P. lycii SKLVPFSGHM tVEKLaC... ....sgkeaV RVLVNDAVQP

Basidio **SKLVPFSARM VVERL-C--- GT-------- ---------V**
RVLVNDAVQP

401 441

P. involutus (*phyA1*) LEFCGGDrNG lCTLAkFVES QtFARsDGaG DFEKCFATSa ~

P. involutus (*phyA2*) LEFCGGDqDG lCALDkFVES QaYARsGGaG DFEKCLATTv ~

T. pubescens LAFCGADtsG vCTLDAFVES QaYARNDGEG DFEKCFAT~~ ~

A. pediades LKFCGGDmDS lCTLEAFVES QkYAREDGQG DFEKCFD~~~ ~

P. lycii LEFCGG.vDG vCeLsAFVES QtYARENGQG DFAKCgfvPs e

Basidio **LEFCGGD-DG -CTLDAFVES Q-YAREDGQG DFEKCFATP- -**

Figure 5

```
                                1
50

   A. terreus 9a1               KhsdCNSVDh GYQCfPELSH kWGlYAPYFS LqDESPFPlD
VPeDCHITFV

   A. terreus cbs               NhsdCtSVDr GYQCfPELSH kWGlYAPYFS LqDESPFPlD
VPdDCHITFV

   A. niger var. awamori NqsTCDTVDq GYQCfSEtSH LWGQYAPFFS LANESAISPD
VPaGCRVTFa

   A. niger NRRL3135            NqsSCDTVDq GYQCfSEtSH LWGQYAPFFS LANESvISPE
VPaGCRVTFa

   A. fumigatus 13073           GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK
LPkDCRITLV

   A. fumigatus 32722           GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK
LPkDCRITLV

   A. fumigatus 58128           GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK
LPkDCRITLV

   A. fumigatus 26906           GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK
LPkDCRITLV

   A. fumigatus 32239           GSkACDTVEl GYQCsPGtSH LWGQYSPFFS LEDElSVSSD
LPkDCRVTFV

   E. nidulans                  QNHSCNTaDG GYQCfPNVSH VWGQYSPYFS IEQESAISeD
VPhGCeVTFV

   T. thermophilus              DSHSCNTVEG GYQCrPEISH sWGQYSPFFS LADQSEISPD
VPqNCKITFV

   T. lanuginosa                ~~~~~~~~~~ ~~~~nvDIAR hWGQYSPFFS LAEvSEISPA
VPkGCRVeFV
```

M. thermophila ESRPCDTpDl GFQCgTAISH FWGQYSPYFS VPsElDaS.. IPdDCeVTFa

Basidio xSxPxrxtAA qLPipxQxqx xWSPYSPYFP VAxyxA.... pPaGCQIxqV

Consensus NSHSCDTVDG GYQC-PEISH LWGQYSPFFS LADESAISPD VP-GCRVTFV

Fcp10 NSHSCDTVDG GYQCFPEISH LWGQYSPFFS LADESAISPD VPKGCRVTFV

51

100

A. terreus 9a1 QVLARHGARs PThSKTKaYA AtIaAIQKSA TaFpGKYAFL QSYNYSLDSE

A. terreus cbs QVLARHGARs PTdSKTKaYA AtIaAIQKNA TaLpGKYAFL KSYNYSMGSE

A. niger var. *awamori* QVLSRHGARY PTeSKGKKYS ALIeEIQQNv TtFDGKYAFL KTYNYSLGAD

A. niger NRRL3135 QVLSRHGARY PTdSKGKKYS ALIeEIQQNA TtFDGKYAFL KTYNYSLGAD

A. fumigatus 13073 QVLSRHGARY PTSSKSKKYk kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus 32722 QVLSRHGARY PTSSKSKKYk kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus 58128 QVLSRHGARY PTSSKSKKYk kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus 26906 QVLSRHGARY PTSSKSKKYk kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus 32239 QVLSRHGARY PTASKSKKYk kLVtAIQKNA TeFKGKFAFL ETYNYTLGAD

E. nidulans QVLSRHGARY PTeSKSKaYS GLIeAIQKNA TsFwGQYAFL ESYNYTLGAD

T. thermophilus QLLSRHGARY PTSSKTElYS qLIsrIQKtA TaYKGyYAFL KdYrYqLGAN

T. lanuginosa QVLSRHGARY PTAhKSEvYA ELLqrIQDtA TeFKGDFAFL RdYaYhLGAD

M. thermophila QVLSRHGARa PTlkRAasYv DLIdrIHhGA isYgPgYEFL RTYDYTLGAD

Basidio NIIqRHGARF PTSGaAtRiq AaVakLQsax xxtDPKLDFL xnxtYxLGxD

Consensus QVLSRHGARY PTSSKSKKYS ALI-AIQKNA T-FKGKYAFL KTYNYTLGAD

Fcp10 QVLSRHGARY PTSSKSKKYS ALIEAIQKNA TAFKGKYAFL KTYNYTLGAD

101

150

A. terreus 9a1 ELTPFGrNQL rDlGaQFYeR YNAL.TRhIn PFVRATDAsR VhESAEKFVE

A. terreus cbs NLTPFGrNQL qDlGaQFYRR YDTL.TRhIn PFVRAADSsR VhESAEKFVE

A. niger var. *awamori* DLTPFGEQEL VNSGIKFYQR YESL.TRnII PFIRSSGSsR VIASGEKFIE

A. niger NRRL3135 DLTPFGEQEL VNSGIKFYQR YESL.TRnIV PFIRSSGSsR VIASGKKFIE

A. fumigatus 13073 DLTPFGEQQL VNSGIKFYQR YKAL.ARsVV PFIRASGSDR VIASGEKFIE

A. fumigatus 32722 DLTPFGEQQL VNSGIKFYQR YKAL.ARsVV PFIRASGSDR VIASGEKFIE

A. fumigatus 58128 DLTPFGEQQL VNSGIKFYQR YKAL.ARsVV PFIRASGSDR VIASGEKFIE

A. fumigatus 26906 DLTAFGEQQL VNSGIKFYQR YKAL.ARsVV PFIRASGSDR VIASGEKFIE

A. fumigatus 32239 DLTPFGEQQM VNSGIKFYQK YKAL.AgsVV PFIRSSGSDR VIASGEKFIE

E. nidulans DLTiFGENQM VDSGaKFYRR YKnL.ARknt PFIRASGSDR VVASAEKFIN

T. thermophilus DLTPFGENQM IQlGIKFYnH YKSL.ARnaV PFVRCSGSDR VIASGrlFIE

T. lanuginosa NLTRFGEEQM MESGrQFYHR YREq.AReIV PFVRAAGSAR VIASAEfFnr

M. thermophila ELTRtGQQQM VNSGIKFYRR YRAL.ARksI PFVRTAGqDR VVhSAENFtQ

Basidio DLvPFGAxQs sQAGqEaFtR YsxLvSxdnL PFVRASGSDR VVDSAtNWtA

Consensus DLTPFGEQQM VNSGIKFYRR YKAL-AR-IV PFVRASGSDR VIASAEKFIE

Fcp10 DLTPFGEQQM VNSGIKFYRR YKAL.ARKIV PFVRASGSDR VIASAEKFIE

151

200

A. terreus 9a1	GFQTARqDDh hAnphQPSPr VDVaIPEGsA YNNTLEHSLC TAFEs...St
A. terreus cbs	GFQNARqGDP hAnphQPSPr VDVVIPEGtA YNNTLEHSIC TAFEa...St
A. niger var. *awamori*	GFQSTKLkDP rAqpgQSSPk IDVVISEAsS sNNTLDpGtC TvFEd...SE
A. niger NRRL3135	GFQSTKLkDP rAqpgQSSPk IDVVISEAsS sNNTLDpGtC TvFEd...SE
A. fumigatus 13073	GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32722	GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 58128	GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 26906	GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32239	GFQqANVADP gAt.nRAAPV ISVIIPESeT YNNTLDHSVC TnFEa...SE
E. nidulans	GFRkAQLhDh g.s.gQATPV VNVIIPEidG FNNTLDHStC vSFEn...dE
T. thermophilus	GFQSAKVlDP hSdkhDAPPt INVIIeEGpS YNNTLDtGsC PvFEd...Ss
T. lanuginosa	GFQdAKdrDP rSnkdQAePV INVIISEEtG sNNTLDgltC PAaEe...Ap
M. thermophila	GFHSAlLADR gStvrPTlPy dmVVIPETaG aNNTLHNDLC TAFEegPySt

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   Basidio              GFaxA..... ..sxntxxPx LxVILSExg. .NDTLDDNMC
.......PxAG

             Consensus  GFQSAKLADP -A---QASPV INVIIPEG-G YNNTLDHGLC
TAFE--P-SE

                 Fcp10  GFQSAKLADP GANPHQASPV INVIIPEGAG YNNTLDHGLC
TAFEE...SE


                        201
250

   A. terreus 9a1       VGDDavANFT AVFAPAIaqR LEAdLPGVQL StDDVVNLMA
MCPFETVSlT

   A. terreus cbs       VGDAaADNFT AVFAPAIakR LEAdLPGVQL SADDVVNLMA
MCPFETVSlT

   A. niger var. awamori LADtVEANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD
MCSFDTIStS

   A. niger NRRL3135    LADtVEANFT AtFvPSIRqR LEndLSGVtL TDtEVtyLMD
MCSFDTIStS

   A. fumigatus 13073   LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD
MCSFDTVArT

   A. fumigatus 32722   LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD
MCSFDTVArT

   A. fumigatus 58128   LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD
MCSFDTVArT

   A. fumigatus 26906   LGDEVAANFT ALFAPdIRAR aKkhLPGVtL TDEDVVSLMD
MCSFDTVArT

   A. fumigatus 32239   LGDEVEANFT ALFAPAIRAR IEkhLPGVQL TDDDVVSLMD
MCSFDTVArT
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E. nidulans rADEIEANFT AIMGPPIRkR LEndLPGIKL TNENVIyLMD MCSFDTMArT

T. thermophilus gGHDaQEKFA kqFAPAIlEK IKDhLPGVDL AvsDVpyLMD LCPFETLArn

T. lanuginosa .DptqpAEFl qVFGPRVlkK ItkhMPGVNL TlEDVplFMD LCPFDTVGsd

M. thermophila IGDDaQDtYl StFAGPItAR VNAnLPGaNL TDADtVaLMD LCPFETVAsS

Basidio dSDpqxnxWl AVFAPPItAR LNAaaPGaNL TDxDaxNLxx LCPFETVS..

Consensus LGDDVEANFT AVFAPPIRAR LEA-LPGVNL TDEDVVNLMD MCPFDTVA-T

Fcp10 LGDDVEANFT AVFAPPIRAR LEAHLPGVNL TDEDVVNLMD MCPFDTVART

251

300

A. terreus 9a1 dD..Aht... ......LSPF CDLFTa..tE WtQYNYLlSL dKYYGYGGGN

A. terreus cbs dD..Aht... ......LSPF CDLFTa..aE WtQYNYLlSL dKYYGYGGGN

A. niger var. awamori Tv..DTK... ......LSPF CDLFTH..dE WiHYDYLQSL kKYYGHGAGN

A. niger NRRL3135 Tv..DTK... ......LSPF CDLFTH..dE WiNYDYLQSL kKYYGHGAGN

A. fumigatus 13073 SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN

A. fumigatus 32722 SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN

A. fumigatus 58128 SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN

A. fumigatus 26906 SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN

A. fumigatus 32239 AD..ASE... ......LSPF CAIFTH..nE WkKYDYLQSL gKYYGYGAGN

E. nidulans AH..GTE... ......LSPF CAIFTE..kE WlQYDYLQSL sKYYGYGAGS

T. thermophilus ht..DT.... ......LSPF CALsTQ..eE WqaYDYYQSL gKYYGnGGGN

T. lanuginosa PvlfPrQ... ......LSPF CHLFTa..dD WmaYDYYyTL dKYYSHGGGS

M. thermophila SsdpATadag ggngrpLSPF CrLFSE..sE WraYDYLQSV gKWYGYGPGN

Basidio ....xexxSxF CDLFexxpeE FxaFxYxgdL dKFYGtGyGQ

Consensus SD--ATQ--- ------LSPF CDLFTH---E W-QYDYLQSL - KYYGYGAGN

Fcp10 SD..ATQ... ......LSPF CDLFTH..DE WIQYDYLQSL GKYYGYGAGN

301

350

A. terreus 9a1 PLGPvQGVGW aNELMARLTR A.PVHDHTCv NNTLDASPAT FPLNATLYAD

A. terreus cbs PLGPvQGVGW aNELIARLTR S.PVHDHTCv NNTLDANPAT FPLNATLYAD

A. niger var. *awamori* PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD

A. niger NRRL3135 PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSSPAT FPLNSTLYAD

A. fumigatus 13073 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus 32722 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus 58128 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus 26906 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus 32239 PLGPAQGIGF tNELIARLTN S.PVQDHTST NsTLDSDPAT FPLNATIYvD

E. nidulans PLGPAQGIGF tNELIARLTQ S.PVQDNTST NHTLDSNPAT FPLDrkLYAD

T. thermophilus PLGPAQGVGF vNELIARMTH S.PVQDYTTv NHTLDSNPAT FPLNATLYAD

T. lanuginosa AFGPSRGVGF vNELIARMTg NlPVKDHTTv NHTLDdNPET FPLDAvLYAD

M. thermophila PLGPTQGVGF vNELLARLA. GvPVRDgTST NRTLDGDPrT FPLGrPLYAD

Basidio PLGPvQGVGY iNELLARLTx qa.VRDNTqT NRTLDSSPxT FPLNrTFYAD

Consensus PLGPAQGVGF -NELIARLTH S-PVQDHTST NHTLDSNPAT FPLNATLYAD

Fcp10 PLGPAQGVGF VNELIARLTH S.PVQDHTST NHTLDSNPAT FPLNATLYAD

351

400

A. terreus 9a1 FSHDSnLVSI FWALGLYNGT aPLSqTSVE. .SvsQTDGYA AAWTVPFAAR

A. terreus cbs FSHDSnLVSI FWALGLYNGT kPLSqTTVE. .ditrTDGYA AAWTVPFAAR

A. niger var. *awamori* FSHDNGIISI LFALGLYNGT kPLSTTTVE. .NitQTDGFS SAWTVPFASR

A. niger NRRL3135 FSHDNGIISI LFALGLYNGT kPLSTTTVE. .NitQTDGFS SAWTVPFASR

A. fumigatus 13073 FSHDNSMVSI FFALGLYNGT ePLSrTSVE. .SaKElDGYS ASWvVPFGAR

A. fumigatus 32722 FSHDNSMVSI FFALGLYNGT gPLSrTSVE. .SaKElDGYS ASWvVPFGAR

A. fumigatus 58128 FSHDNSMVSI FFALGLYNGT ePLSrTSVE. .SaKElDGYS ASWvVPFGAR

A. fumigatus 26906 FSHDNSMVSI FFALGLYNGT ePLSrTSVE. .SaKElDGYS ASWvVPFGAR

A. fumigatus 32239 FSHDNGMIPI FFAMGLYNGT ePLSqTSeE. .StKESNGYS ASWAVPFGAR

E. nidulans FSHDNSMISI FFAMGLYNGT qPLSmdSVE. .SiQEmDGYA ASWTVPFGAR

T. thermophilus FSHDNTMtSI FaALGLYNGT akLSTTeIK. .SiEETDGYS AAWTVPFGGR

T. lanuginosa FSHDNTMtGI FsAMGLYNGT kPLSTSkIQP pTgAAADGYA ASWTVPFAAR

M. thermophila FSHDNdMMGV LgALGaYDGv pPLdkTA..R rdpEElGGYA ASWAVPFAAR

Basidio FSHDNqMVAI FsAMGLFNqS aPLdPSxpDP nrt.....Wv TSklVPFSAR

Consensus FSHDNTMVSI FFALGLYNGT -PLSTTSVEP -S-EETDGYA ASWTVPFAAR

Fcp10 FSHDNTMVSI FFALGLYNGT KPLSTTSVE. .SIEETDGYA ASWTVPFAAR

401

450

A. terreus 9a1 AYVEMMQC.. ra..... .....EKEPL VRVLVNDRVM PLHGCPtDKL

A. terreus cbs AYIEMMQC.. ra..... .....EKQPL VRVLVNDRVM PLHGCAVDNL

A. niger var. *awamori* lYVEMMQC.. Qa..... .....EQEPL VRVLVNDRVV PLHGCPIDaL

A. niger NRRL3135 lYVEMMQC.. Qa..... .....EQEPL VRVLVNDRVV PLHGCPVDaL

A. fumigatus 13073 AYfEtMQC.. Ks..... .....EKEPL VRaLINDRVV PLHGCDVDKL

A. fumigatus 32722 AYfEtMQC.. Ks..... .....EKEPL VRaLINDRVV PLHGCDVDKL

A. fumigatus 58128 AYfEtMQC.. Ks..... .....EKESL VRaLINDRVV PLHGCDVDKL

A. fumigatus 26906 AYfEtMQC.. Ks..... .....EKEPL VRaLINDRVV PLHGCDVDKL

A. fumigatus 32239 AYfEtMQC.. Ks..... .....EKEPL VRaLINDRVV PLHGCAVDKL

E. nidulans AYfELMQC.. E..... .....KKEPL VRVLVNDRVV PLHGCAVDKF

T. thermophilus AYIEMMQC.. Dd..... .....sDEPV VRVLVNDRVV PLHGCEVDsL

T. lanuginosa AYVELLRC.. Etetsseeee EG...EDEPF VRVLVNDRVV PLHGCrVDRW

M. thermophila iYVEkMRC.. sgggggggg EGrqeKDEeM VRVLVNDRVM TLkGCGaDEr

Basidio mvVErLxCxx xgtxxxxxxx xxxxxxxxx VRVLVNDaVq PLEfCGgDxd

Consensus AYVEMMQC-- E--------- EG---EKEPL VRVLVNDRVV PLHGCGVDKL

Fcp10 AYVEMMQC.. EA..... .....EKEPL VRVLVNDRVV PLHGCGVDKL

451 482

A. terreus 9a1 GRCKrDAFVA GLSFAQAG.. GNWADCF~~~ ~~

A. terreus cbs GRCKrDDFVE GLSFARAG.. GNWAECF~~~ ~~

A. niger var. *awamori* GRCtrDsFVr GLSFARSG.. GDWAECsA~~ ~~

A. niger NRRL3135 GRCtrDsFVr GLSFARSG.. GDWAECFA~~ ~~

A. fumigatus 13073 GRCKlNDFVK GLSWARSG.. GNWGECFS~~ ~~

A. fumigatus 32722 GRCKlNDFVK GLSWARSG.. GNWGECFS~~ ~~

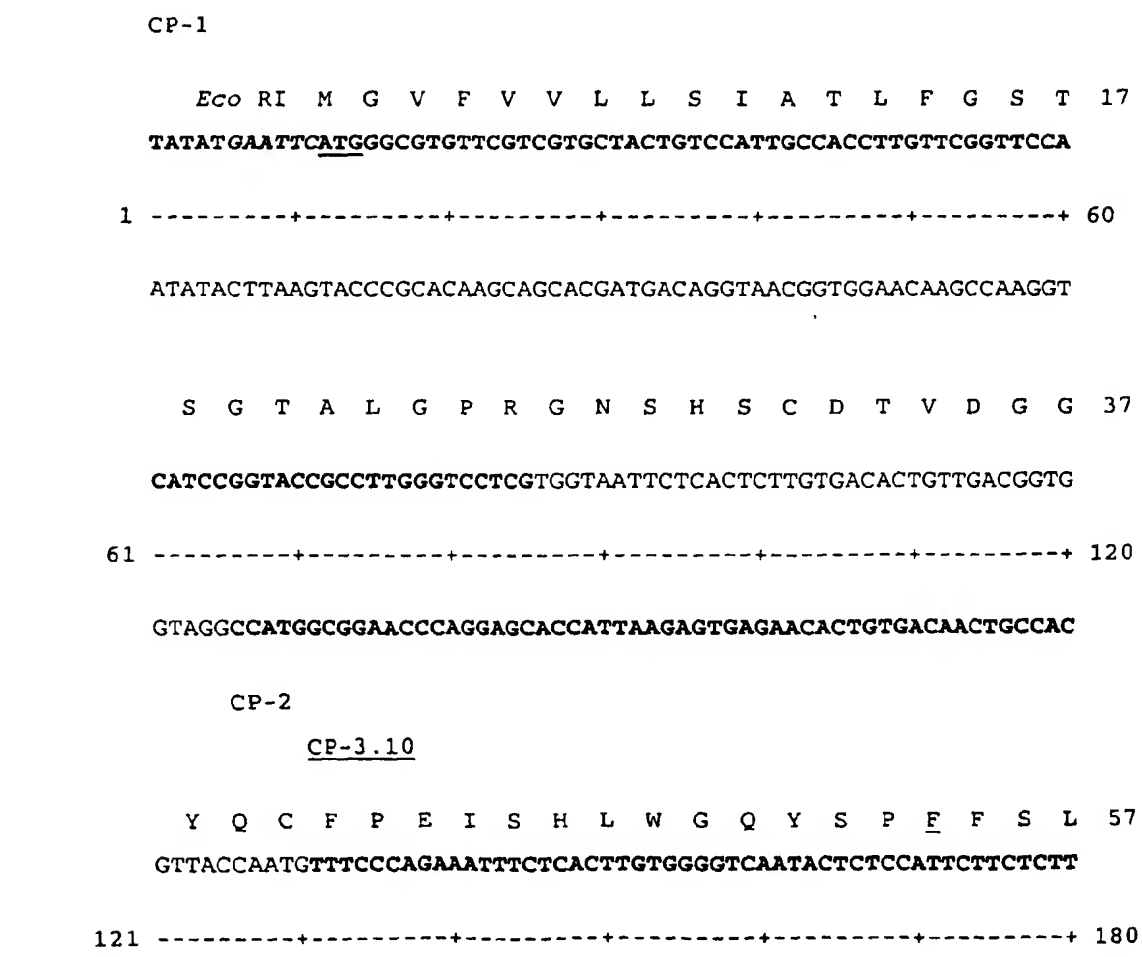
A. fumigatus 58128 GRCKlNDFVK GLSWARSG.. GNWGECFS~~ ~~

A. fumigatus 26906 GRCKlNDFVK GLSWARSG.. GNWGECFS~~ ~~

A. fumigatus 32239 GRCKlKDFVK GLSWARSG.. GNSEQSFS~~ ~~

E. nidulans	GRCtlDDWVE GLNFARSG.. GNWKtCFTl~ ~~
T. thermophilus	GRCKrDDFVr GLSFARqG.. GNWEGCYAas e~
T. lanuginosa	GRCRrDEWIK GLTFARqG.. GHWDrCF~~~ ~~
M. thermophila	GmCtlErFIE SMAFARGN.. GKWDlCFA~~ ~~
Basidio	GxCtlDAFVE SqxYAReDgq GDFEKCFAtp xx
Consensus	GRCK-DDFVE GLSFARSG-- GNWEECFA-- --
Fcp10	**GRCKRDDFVE GLSFARSG.. GNWEECFA.. ..**

Figure 6



CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTAAGAAGAGAA

A D E S A I S P D V P K G C R V T F V Q 77

TGGCTGACGAATCTGCTATTTCTCCAGACGTTCCAAAGGGTTGTAGAGTTACTTTCGTTC

181 ---------+---------+---------+---------+---------+---------+ 240

ACCGACTGCTTAGAC**GATAAAGAGGTCTGCAAGGTTTCCCGACATCTCAATGAAAGCAAG**

CP-4.10

CP-5.10

V L S R H G A R Y P T S S K S K K Y S A 97

AAGTTTTGTCTAGACACGGT**GCTAGATACCCAACTTCTTCTAAGTCTAAGAAGTACTCTG**

241 ---------+---------+---------+---------+---------+---------+ 300

TTCAAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTCAGATTCTTCATGAGAC

L I E A I Q K N A T A F K G K Y A F L K 117

CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA

301 ---------+---------+---------+---------+---------+---------+ 360

GAAACTAACTTCGATAAGTTTTCTT**GCGATGACGAAAGTTCCCATTCATGCGAAAGAACT**

CP-6

CP-7.10

T Y N Y T L G A D D L T P F G E Q Q M V 137

AGACTTACAACTACACTTTGGGTGCTGACG**ACTTGACTCCATTCGGTGAACAACAAATGG**

361 ---------+---------+---------+---------+---------+---------+ 420

TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACC

N S G I K F Y R R Y K A L A R K I V P F 157

```
    TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT

421 ---------+---------+---------+---------+---------+---------+ 480

    AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA

                                        CP-8.10

                                            CP-9.10

      V  R  A  S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F 177
    TCGTTAGAGCTTCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT
481 ---------+---------+---------+---------+---------+---------+ 540
    AGCAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA


      Q  S  A  K  L  A  D  P  G  A  N  P  H  Q  A  S  P  V  I  N 197
    TCCAATCTGCTAAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTA

541 ---------+---------+---------+---------+---------+---------+ 600

    AGGTTAGACGATTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAAT

                                                CP-10.10

                                                    CP-11.10

      V  I  I  P  E  G  A  G  Y  N  N  T  L  D  H  G  L  C  T  A 217
    ACGTTATTATTCCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTG

601 ---------+---------+---------+---------+---------+---------+ 660

    TGCAATAATAAGGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGAC


      F  E  E  S  E  L  G  D  D  V  E  A  N  F  T  A  V  F  A  P 237

    CTTTCGAAGAATCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTC

661 ---------+---------+---------+---------+---------+---------+ 720

    GAAAGCTTCTTAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAG
```

CP-12.10

P I R A R L E A H L P G V N L T D E D V 257

CACCTATTAGAGCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACG

721 ---------+---------+---------+---------+---------+---------+ 780

GTGGATAATCTCGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGC

CP-13.10

V N L M D M C P F D T V A R T S D A T Q 277

TTGTTAACTTGATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTC

781 ---------+---------+---------+---------+---------+---------+ 840

AACAATTGAACTACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAG

L S P F C D L F T H D E W I Q Y D Y L Q 297

AATTGTCTCCATTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGC

841 ---------+---------+---------+---------+---------+---------+ 900

TTAAC**AGAGGTAAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACG**

CP-14.10

CP-15.10

S L G K Y Y G Y G A G N P L G P A Q G V 317

AATCTTTGGG**TAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG**

901 ---------+---------+---------+---------+---------+---------+ 960

TTAGAAACCCATTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCAC

G F V N E L I A R L T H S P V Q D H T S 337

TTGGTTTCGTTAACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTT

961 ---------+---------+---------+---------+---------+---------+
1020

AACCAAAGCAATTGC**TTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAA**

CP-16.10
CP-17.10

T N H T L D S N P A T F P L N A T L Y A 357

CTACTAACCACACTTTGGAC**TCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG**

1021 ---------+---------+---------+---------+---------+---------+
1080

GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

D F S H D N T M V S I F F A L G L Y N G 377

CTGACTTCTCTCACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACG

1081 ---------+---------+---------+---------+---------+---------+
1140

GACTGAAGAGAGTGCTGTTGTGATA**CCAAAGATAAAAGAAGCGAAACCCAAACATGTTGC**

CP-18.10
CP-19.10

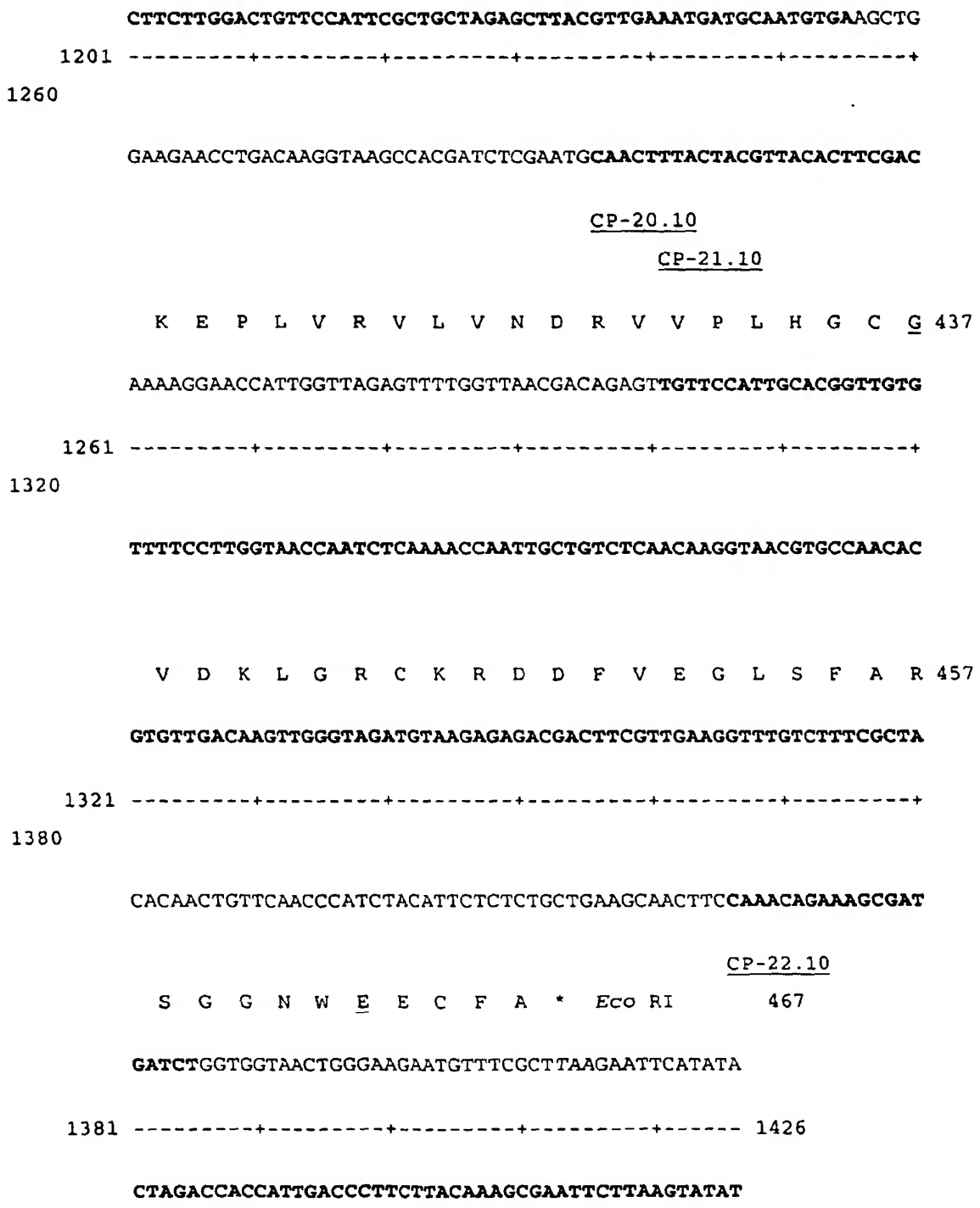
T K P L S T T S V E S I E E T D G Y A A 397

GTACTAAGCCATTGTCTACTACTTCTGTTG**AATCTATTGAAGAAACTGACGGTTACGCTG**

1141 ---------+---------+---------+---------+---------+---------+
1200

CATGATTCGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGCGAC

S W T V P F A A R A Y V E M M Q C E A E 417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1201 ---------+---------+---------+---------+---------+---------+
1260
GAAGAACCTGACAAGGTAAGCCACGATCTCGAATGCAACTTTACTACGTTACACTTCGAC
CP-20.10
CP-21.10
K E P L V R V L V N D R V V P L H G C G 437
AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 ---------+---------+---------+---------+---------+---------+
1320
TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC
V D K L G R C K R D D F V E G L S F A R 457
GTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA
1321 ---------+---------+---------+---------+---------+---------+
1380
CACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
CP-22.10
S G G N W E E C F A * Eco RI 467
GATCTGGTGGTAACTGGGAAGAATGTTTCGCTTAAGAATTCATATA
1381 ---------+---------+---------+---------+------ 1426
CTAGACCACCATTGACCCTTCTTACAAAGCGAATTCTTAAGTATAT

Figure 7

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                                1
50

P. involutus (phyA1)      ~~~~~~~~~~ ~FPipeseqR nWSPYSPYFP LAEykA....
pPaGCQInqV

P. involutus (phyA2)      ~~~~~~~~~~ ~FsipeseqR nWSPYSPYFP LAEykA....
pPaGCeInqV

T. pubescens              ~~~~~~~~~~ ~LDvtRDVqQ sWSmYSPYFP aAtyvA....
pPaSCQInqV

A. pediades               ~~~~~~~~~~ ~pffpPQIqD sWAaYTPYYP VqAyTP....
pPKDCKITqV

P. lycii                  ~~~~~~~~~~ ~LPipAQnTs nWGPYdPFFP VEpyAA....
pPEGCtVTqV

A. terreus 9a1            KhsdCNSVDh GYQCfPELSH kWGlYAPYFS LqDESPFPlD
VPEDCHITFV

A. terreus cbs            NhsdCtSVDr GYQCfPELSH kWGlYAPYFS LqDESPFPlD
VPDDCHITFV

A. niger var. awamori     NqsTCDTVDq GYQCfSEtSH LWGQYAPFFS LANESAISPD
VPaGCRVTFa

A. niger T213             NqsSCDTVDq GYQCfSEtSH LWGQYAPFFS LANESvISPD
VPaGCRVTFa

A. niger NRRL3135         NqsSCDTVDq GYQCfSEtSH LWGQYAPFFS LANESvISPE
VPaGCRVTFa

A. fumigatus ATCC13073    GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK
LPKDCRITLV

A. fumigatus ATCC32722    GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK
LPKDCRITLV

A. fumigatus ATCC58128    GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK
LPKDCRITLV
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A. fumigatus ATCC26906 GSkSCDTVDl GYQCsPAtSH LWGQYSPFFS LEDElSVSSK LPKDCRITLV

A. fumigatus ATCC32239 GSkACDTVEl GYQCsPGtSH LWGQYSPFFS LEDElSVSSD LPKDCRVTFV

E. nidulans QNHSCNTaDg GYQCfPNVSH VWGQYSPYFS IEQESAISeD VPhGCeVTFV

T. thermophilus DSHSCNTVEg GYQCrPEISH sWGQYSPFFS LADQSEISPD VPQNCKITFV

T. lanuginosa ~~~~~~~~~~ ~~~~nvDIAR hWGQYSPFFS LAEvSEISPA VPKGCRVeFV

M. thermophila ESRPCDTpDl GFQCgTAISH FWGQYSPYFS VPsElDaS.. IPDDCeVTFa

Consensus Seq. 11 NSHSCDTVD- GYQC-PEISH LWGQYSPFFS LADESAISPD VPKGCRVTFV

51

100

P. involutus (phyA1) NIIqRHGARF PTSGaTtRik AgLtKLQgvq nftDAKFnFI KSFKYdLGns

P. involutus (phyA2) NIIqRHGARF PTSGaAtRik AgLsKLQsvq nftDPKFDFI KSFtYdLGTs

T. pubescens HIIqRHGARF PTSGaAKRiq TaVAKLKaaS nytDPlLAFV tnYtYSLGqD

A. pediades NIIqRHGARF PTSGaGtRiq AaVKKLQsak TytDPRLDFL tnYtYTLGhD

P. lycii NLIqRHGARW PTSGarsRqv AaVAKIQmar PftDPKYEFL NdFvYkFGvA

A. terreus 9a1 QVLARHGARs PThSKTKaYA AtIAaIQKSA TaFpGKYAFL QSYNYSLDSE

A. terreus cbs QVLARHGARs PTdSKTKaYA AtIAaIQKNA TaLpGKYAFL KSYNYSMGSE

A. niger var. *awamori* QVLSRHGARY PTeSKGKKYS ALIEeIQQNv TtFDGKYAFL KTYNYSLGAD

A. niger T213 QVLSRHGARY PTeSKGKKYS ALIEeIQQNv TtFDGKYAFL KTYNYSLGAD

A. niger NRRL3135 QVLSRHGARY PTdSKGKKYS ALIEeIQQNA TtFDGKYAFL KTYNYSLGAD

A. fumigatus ATCC13073 QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus ATCC32722 QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus ATCC58128 QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus ATCC26906 QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL KTYNYTLGAD

A. fumigatus ATCC32239 QVLSRHGARY PTASKSKKYk kLVtaIQKNA TeFKGKFAFL ETYNYTLGAD

E. nidulans QVLSRHGARY PTeSKSKaYS GLIEaIQKNA TsFwGQYAFL ESYNYTLGAD

T. thermophilus QLLSRHGARY PTSSKTElYS qLIsRIQKtA TaYKGyYAFL KdYrYqLGAN

T. lanuginosa QVLSRHGARY PTAhKSEvYA ELLQRIQDtA TeFKGDFAFL RdYaYhLGAD

M. thermophila QVLSRHGARa PTlkRAasYv DLIDRIHhGA isYgPgYEFL RTYDYTLGAD

Consensus Seq. 11 **QVLSRHGARY PTSSKSKKYS ALIERIQKNA T-FKGKYAFL KTYNYTLGAD**

101

150

P. involutus (*phyA1*) DLvPFGAaQs fDAGqEaFaR YskLvSKNnL PFIRAdGSDR VVDSAtNWtA

P. involutus (*phyA2*) DLvPFGAaQs fDAGLEvFaR YskLvSsDnL PFIRSdGSDR VVDTAtNWtA

T. pubescens sLveLGAtQs sEAGqEaFtR YsSLvSaDeL PFVRASGSDR VVATANNWtA

A. pediades DLvPFGAlQs sQAGeEtFQR YsfLvSKEnL PFVRASSSNR VVDSAtNWtE

P. lycii DLlPFGANQs hQTGtDMYtR YsTLfEgGdV PFVRAAGdQR VVDSStNWtA

A. terreus 9a1 ELTPFGrNQL rDlGaQFYeR YNAL.TRHIn PFVRATDAsR VhESAEKFVE

A. terreus cbs NLTPFGrNQL qDlGaQFYRR YDTL.TRHIn PFVRAADSsR VhESAEKFVE

A. niger var. *awamori* DLTPFGEQEL VNSGIKFYQR YESL.TRNII PFIRSSGSsR VIASGEKFIE

A. niger T213 DLTPFGEQEL VNSGIKFYQR YESL.TRNII PFIRSSGSsR VIASGEKFIE

A. niger NRRL3135 DLTPFGEQEL VNSGIKFYQR YESL.TRNIV PFIRSSGSsR VIASGKKFIE

A. fumigatus ATCC13073 DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV PFIRASGSDR VIASGEKFIE

A. fumigatus ATCC32722 DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV PFIRASGSDR VIASGEKFIE

A. fumigatus ATCC58128 DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV PFIRASGSDR VIASGEKFIE

A. fumigatus ATCC26906 DLTAFGEQQL VNSGIKFYQR YKAL.ARSVV PFIRASGSDR VIASGEKFIE

A. fumigatus ATCC32239 DLTPFGEQQM VNSGIKFYQK YKAL.AgSVV PFIRSSGSDR VIASGEKFIE

E. nidulans DLTiFGENQM VDSGaKFYRR YKnL.ARKnt PFIRASGSDR VVASAEKFIN

T. thermophilus DLTPFGENQM IQlGIKFYnH YKSL.ARNaV PFVRCSGSDR VIASGrlFIE

T. lanuginosa NLTRFGEEQM MESGrQFYHR YREq.AREIV PFVRAAGSAR VIASAEfFnr

M. thermophila ELTRtGQQQM VNSGIKFYRR YRAL.ARKsI PFVRTAGqDR VVhSAENFtQ

Consensus Seq. 11 DLTPFGENQM VNSGIKFYRR YKAL-ARNIV PFVRASGSDR VIASAEKFIE

151

200

P. involutus (*phyA1*) GFaSA..... ..shNtvqPk LNLILPQ..T gNDTLEDNMC PAaGD.....

P. involutus (*phyA2*) GFaSA..... ..srNaiqPk LDLILPQ..T gNDTLEDNMC PAaGE.....

T. pubescens GFalA..... ..ssNsiTPV LSVIISE..A gNDTLDDNMC PAaGD.....

A. pediades GFsAA..... ..shHvlNPI LfVILSE..S LNDTLDDAMC PnaGs.....

P. lycii GFgdA..... ..sgEtvlPt LQVVLQE..E gNcTLcNNMC
PnevD.....

A. terreus 9a1 GFQTARqDDh hAnpHQPSPr VDVaIPEGSA YNNTLEHSLC
TAFEs...ST

A. terreus cbs GFQNARqGDP hAnpHQPSPr VDVVIPEGTA YNNTLEHSIC
TAFEA...ST

A. niger var. *awamori* GFQSTKLkDP rAqpgQSSPk IDVVISEASS sNNTLDpGtC
TvFED...Se

A. niger T213 GFQSTKLkDP rAqpgQSSPk IDVVISEASS sNNTLDpGtC
TvFED...Se

A. niger NRRL3135 GFQSTKLkDP rAqpgQSSPk IDVVISEASS sNNTLDpGtC
TvFED...Se

A. fumigatus ATCC13073 GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC
TkFEA...Sq

A. fumigatus ATCC32722 GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC
TkFEA...Sq

A. fumigatus ATCC58128 GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC
TkFEA...Sq

A. fumigatus ATCC26906 GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC
TkFEA...Sq

A. fumigatus ATCC32239 GFQqANVADP gAt.NRAAPV ISVIIPESeT YNNTLDHSVC
TnFEA...Se

E. nidulans GFRkAQLhDh g.s.gQATPV VNVIIPEidG FNNTLDHStC
vSFEN...de

T. thermophilus GFQSAKVlDP hSdkHDAPPt INVIIeEGPS YNNTLDtGsC
PvFED...SS

T. lanuginosa GFQdAKdrDP rSnkDQAePV INVIISEETG sNNTLDgltC
PAaEE...AP

M. thermophila GFHSAlLADR gStvRPTlPy dmVVIPETAG aNNTLHNDLC TAFEEgpyST

Consensus Seq. 11 **GFQSAKLADP -A--HQASPV INVIIPEGSG YNNTLDHGLC TAFED---ST**

201

250

P. involutus (*phyA1*) .SDpqvnaWl AVafPSItAR LNAaaPSVNL TDtDafNLVs LCAFlTVSK.

P. involutus (*phyA2*) .SDpqvDaWl AsafPSVtAQ LNAaaPGaNL TDADafNLVs LCPFmTVSK.

T. pubescens .SDpqvnQWl AqFAPPMtAR LNAgaPGaNL TDtDtyNLLt LCPFETVAt.

A. pediades .SDpqtGiWT SIYGTPIanR LNqqaPGaNI TAADVsNLIp LCAFETIvK.

P. lycii .GDESt.tWl GVFAPnItAR LNAaaPSaNL SDsDaLtLMD MCPFDTLSs.

A. terreus 9a1 VGDDAvANFT AVFAPAIaqR LEAdLPGVQL StDDVVNLMA MCPFETVSlT

A. terreus cbs VGDAAADNFT AVFAPAIakR LEAdLPGVQL SADDVVNLMA MCPFETVSlT

A. niger var. *awamori* LADtvEANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIStS

A. niger T213 LADtvEANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIStS

A. niger NRRL3135 LADtvEANFT AtFvPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIStS

A. fumigatus ATCC13073 LGDEvAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVART

A. fumigatus ATCC32722 LGDEvAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVART

A. fumigatus ATCC58128 LGDEvAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVART

A. fumigatus ATCC26906 LGDEvAANFT ALFAPdIRAR aKkhLPGVtL TDEDVVSLMD MCSFDTVART

A. fumigatus ATCC32239 LGDEvEANFT ALFAPAIRAR IEkhLPGVQL TDDDVVSLMD MCSFDTVART

E. nidulans rADEiEANFT AIMGPPIRkR LEndLPGIKL TNENVIyLMD MCSFDTMART

T. thermophilus gGHDAQEKFA kqFAPAIlEK IKDhLPGVDL AvsDVpyLMD LCPFETLARn

T. lanuginosa .DptqpAEFl qVFGPRVlkK ItkhMPGVNL TlEDVplFMD LCPFDTVGsd

M. thermophila IGDDAQDtYl StFAGPItAR VNAnLPGaNL TDADtVaLMD LCPFETVAsS

Consensus Seq. 11 LGDDAEANFT AVFAPPIRAR LEA-LPGVNL TDEDVVNLMD MCPFDTVART

251

300

P. involutus (*phyA1*) ....ekkSdF CtLFegiPGs FeaFAYggdL dKFYGtGyGQ

P. involutus (*phyA2*) ....eqkSdF CtLFegiPGs FeaFAYagdL dKFYGtGyGQ

T. pubescens ....errSeF CDIYeelqAE .daFAYnadL
dKFYGtGyGQ

A. pediades ....etpSPF CNLF..TPEE FaQFEYFgdL
dKFYGtGyGQ

P. lycii ....gnaSPF CDLF..TAEE YvsYEYYydL
dKYYGtGPGN

A. terreus 9a1 dD..Aht... ......LSPF CDLF..TAtE WtQYNYL1SL
dKYYGYGGGN

A. terreus cbs dD..Aht... ......LSPF CDLF..TAAE WtQYNYL1SL
dKYYGYGGGN

A. niger var. *awamori* Tv..DTK... ......LSPF CDLF..ThDE WiHYDYLQSL
kKYYGHGAGN

A. niger T213 Tv..DTK... ......LSPF CDLF..ThDE WiHYDYLRSL
kKYYGHGAGN

A. niger NRRL3135 Tv..DTK... ......LSPF CDLF..ThDE WiNYDYLQSL
kKYYGHGAGN

A. fumigatus ATCC13073 SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL
gKYYGYGAGN

A. fumigatus ATCC32722 SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL
gKYYGYGAGN

A. fumigatus ATCC58128 SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL
gKYYGYGAGN

A. fumigatus ATCC26906 SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL
gKYYGYGAGN

A. fumigatus ATCC32239 AD..ASE... ......LSPF CAIF..ThNE WkKYDYLQSL
gKYYGYGAGN

E. nidulans AH..GTE... ......LSPF CAIF..TEKE W1QYDYLQSL
sKYYGYGAGS

T. thermophilus ht..DT.... ......LSPF CALs..TqEE WqaYDYYQSL gKYYGnGGGN

T. lanuginosa PvlfPrQ... ......LSPF CHLF..TADD WmaYDYYyTL dKYYSHGGGS

M. thermophila SsdpATadag ggngrpLSPF CrLF..SEsE WraYDYLQSV gKWYGYGPGN

Consensus Seq. 11 **SD--ATQ--- ------LSPF CDLF--TADE W-QYDYLQSL - KYYGYGAGN**

301

350

P. involutus (phyA1) eLGPvQGVGY vNELIARLTN S.AVRDNTqT NRTLDASPvT FPLNkTFYAD

P. involutus (phyA2) ALGPvQGVGY iNELLARLTN S.AVNDNTqT NRTLDAaPDT FPLNkTMYAD

T. pubescens PLGPvQGVGY iNELIARLTa q.nVsDHTqT NsTLDSSPET FPLNrTLYAD

A. pediades PLGPvQGVGY iNELLARLTE m.PVRDNTqT NRTLDSSPlT FPLDrSIYAD

P. lycii ALGPvQGVGY vNELLARLTg q.AVRDETqT NRTLDSDPAT FPLNrTFYAD

A. terreus 9a1 PLGPvQGVGW aNELMARLTR A.PVHDHTCv NNTLDASPAT FPLNATLYAD

A. terreus cbs PLGPvQGVGW aNELIARLTR S.PVHDHTCv NNTLDANPAT FPLNATLYAD

A. niger var. *awamori* PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD

A. niger T213 PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD

A. niger NRRL3135 PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSSPAT FPLNSTLYAD

A. fumigatus ATCC13073 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus ATCC32722 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus ATCC58128 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus ATCC26906 PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD

A. fumigatus ATCC32239 PLGPAQGIGF tNELIARLTN S.PVQDHTST NsTLDSDPAT FPLNATIYvD

E. nidulans PLGPAQGIGF tNELIARLTQ S.PVQDNTST NHTLDSNPAT FPLDrkLYAD

T. thermophilus PLGPAQGVGF vNELIARMTH S.PVQDYTTv NHTLDSNPAT FPLNATLYAD

T. lanuginosa AFGPSRGVGF vNELIARMTg NlPVKDHTTv NHTLDdNPET FPLDAvLYAD

M. thermophila PLGPTQGVGF vNELLARLA. GvPVRDgTST NRTLDGDPrT FPLGrPLYAD

Consensus Seq. 11 PLGPAQGVGF -NELIARLTH S-PVQDHTST NHTLDSNPAT FPLNATLYAD

351

400

P. involutus (*phyA1*) FSHDNlMVAV FsAMGLFrqP aPLSTSvpNP wrt.....Wr TSSlVPFSGR

P. involutus (*phyA2*) FSHDNlMVAV FsAMGLFrqS aPLSTSTpDP nrt.....Wl TSSvVPFSAR

T. pubescens FSHDNqMVAI FsAMGLFNqS aPLdPTTpDP art.....Fl vkkiVPFSAR

A. pediades LSHDNqMIAI FsAMGLFNqS sPLdPSfpNP krt.....Wv TSRltPFSAR

P. lycii FSHDNTMVPI FaALGLFNAT a.LdPlkpDe nrl.....Wv DSklVPFSGH

A. terreus 9a1 FSHDSnLVSI FWALGLYNGT aPLSqTSVES Vs..QTDGYA AAWTVPFAAR

A. terreus cbs FSHDSnLVSI FWALGLYNGT KPLSqTTVEd It..rTDGYA AAWTVPFAAR

A. niger var. *awamori* FSHDNGIISI LFALGLYNGT KPLSTTTVEN It..QTDGFS SAWTVPFASR

A. niger T213 FSHDNGIISI LFALGLYNGT KPLSTTTVEN It..QTDGFS SAWTVPFASR

A. niger NRRL3135 FSHDNGIISI LFALGLYNGT KPLSTTTVEN It..QTDGFS SAWTVPFASR

A. fumigatus ATCC13073 FSHDNSMVSI FFALGLYNGT EPLSrTSVES ak..ElDGYS ASWvVPFGAR

A. fumigatus ATCC32722 FSHDNSMVSI FFALGLYNGT gPLSrTSVES ak..ElDGYS ASWvVPFGAR

A. fumigatus ATCC58128 FSHDNSMVSI FFALGLYNGT EPLSrTSVES ak..ElDGYS ASWvVPFGAR

A. fumigatus ATCC26906 FSHDNSMVSI FFALGLYNGT EPLSrTSVES ak..ElDGYS ASWvVPFGAR

A. fumigatus ATCC32239 FSHDNGMIPI FFAMGLYNGT EPLSqTSeES tk..ESNGYS ASWAVPFGAR

E. nidulans FSHDNSMISI FFAMGLYNGT QPLSmdSVES Iq..EmDGYA ASWTVPFGAR

T. thermophilus FSHDNTMtSI FaALGLYNGT akLSTTeIKS Ie..ETDGYS AAWTVPFGGR

T. lanuginosa FSHDNTMtGI FsAMGLYNGT KPLSTSkIQP ptgaAADGYA ASWTVPFAAR

M. thermophila FSHDNdMMGV LgALGaYDGv pPLdkTArrd ..peElGGYA ASWAVPFAAR

Consensus Seq. 11 FSHDNTMVSI FFALGLYNGT KPLSTTSVES I---ETDGYA ASWTVPFAAR

401

450

P. involutus (*phyA1*) mvVErLsC.. fGt..... .....Tk VRVLVQDQVq PLEfCGgDRn

P. involutus (*phyA2*) maVErLsC.. AGt..... .....Tk VRVLVQDQVq PLEfCGgDQd

T. pubescens mvVErLDC.. GGa..... .....Qs VRLLVNDaVq PLafCGaDts

A. pediades mvtErL1CQr DGtGsGGpsr imrNgnvQTF VRILVNDaLq PLkfCGgDmd

P. lycii mtVEkLaC.. ....sgKea VRVLVNDaVq PLEfCGg.vd

A. terreus 9a1 AYVEMMQCrA ....EK...EPL VRVLVNDRVM PLHGCPtDKL

A. terreus cbs AYIEMMQCrA ....EK...QPL VRVLVNDRVM PLHGCAVDNL

A. niger var. *awamori* lYVEMMQCQA ....EQ...EPL VRVLVNDRVV PLHGCPIDaL

A. niger T213 lYVEMMQCQA ....EQ...EPL VRVLVNDRVV PLHGCPIDaL

A. niger NRRL3135 lYVEMMQCQA ....EQ...EPL VRVLVNDRVV PLHGCPVDaL

A. fumigatus ATCC13073 AYfEtMQCKS ....EK...EPL VRaLINDRVV PLHGCDVDKL

A. fumigatus ATCC32722 AYfEtMQCKS ....EK...EPL VRaLINDRVV PLHGCDVDKL

A. fumigatus ATCC58128 AYfEtMQCKS ....EK...ESL VRaLINDRVV PLHGCDVDKL

A. fumigatus ATCC26906 AYfEtMQCKS ....EK...EPL VRaLINDRVV PLHGCDVDKL

A. fumigatus ATCC32239 AYfEtMQCKS ....EK...EPL VRaLINDRVV PLHGCAVDKL

E. nidulans AYfELMQCE. ....KK...EPL VRVLVNDRVV PLHGCAVDKF

T. thermophilus AYIEMMQCDD ....sD...EPV VRVLVNDRVV PLHGCEVDsL

T. lanuginosa AYVELLRCET ETsSeEEeEG ..ED...EPF VRVLVNDRVV PLHGCrVDRW

M. thermophila iYVEkMRCsG GGgGgGGgEG ..rQekdEeM VRVLVNDRVM TLkGCGaDEr

Consensus Seq. 11 AYVEMMQCEA GG-G-GG-EG --EK---EPL VRVLVNDRVV PLHGCGVDKL

	451	482
P. involutus (*phyA1*)	GlCtLAKFVE SqTFARSDga GDFEKCFAts a~	
P. involutus (*phyA2*)	GlCaLDKFVE SqAYARSGga GDFEKCLAtt v~	
T. pubescens	GvCtLDAFVE SqAYARNDge GDFEKCFAt~ ~~	
A. pediades	SlCtLEAFVE SqkYAReDgq GDFEKCFD~~ ~~	
P. lycii	GvCELsAFVE SqTYAReNgq GDFAKCgfvp se	
A. terreus 9a1	GRCKrDAFVA GLSFAQAG.. GNWADCF~~~ ~~	
A. terreus cbs	GRCKrDDFVE GLSFARAG.. GNWAECF~~~ ~~	
A. niger var. *awamori*	GRCtrDsFVr GLSFARSG.. GDWAECsA~~ ~~	
A. niger T213	GRCtrDsFVr GLSFARSG.. GDWAECFA~~ ~~	
A. niger NRRL3135	GRCtrDsFVr GLSFARSG.. GDWAECFA~~ ~~	
A. fumigatus ATCC13073	GRCKLNDFVK GLSWARSG.. GNWGECFS~~ ~~	
A. fumigatus ATCC32722	GRCKLNDFVK GLSWARSG.. GNWGECFS~~ ~~	
A. fumigatus ATCC58128	GRCKLNDFVK GLSWARSG.. GNWGECFS~~ ~~	
A. fumigatus ATCC26906	GRCKLNDFVK GLSWARSG.. GNWGECFS~~ ~~	
A. fumigatus ATCC32239	GRCKLKDFVK GLSWARSG.. GNSEQSFS~~ ~~	
E. nidulans	GRCtLDDWVE GLNFARSG.. GNWktCFTl~ ~~	
T. thermophilus	GRCKrDDFVr GLSFARqG.. GNWEGCYAas e~	
T. lanuginosa	GRCRrDEWIK GLTFARqG.. GHWDrCF~~~ ~~	
M. thermophila	GmCtLErFIE SMAFARGN.. GKWDlCFA~~ ~~	
Consensus Seq. 11	**GRCKLDDFVE GLSFARSG-- GNWAECFA-- --**	

Figure 8

```
          M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T
20

          ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC

        1 ---+---------+---------+---------+---------+---------+---------+------
60

          TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

          A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  Y  Q  C
40

          GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT

       61 ---+---------+---------+---------+---------+---------+---------+------
120

          CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

          F  P  E  I  S  H  L  W  G  T  Y  S  P  Y  F  S  L  A  D  E
60

          TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA

      121 ---+---------+---------+---------+---------+---------+---------+------
180

          AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT

          S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q  V  L  S
80

          TCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTCAAGTTTTGTCT

      187 ---+---------+---------+---------+---------+---------+---------+------
240
```

```
        AGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAGTTCAAAACAGA

        R  H  G  A  R  Y  P  T  S  S  A  S  K  A  Y  S  A  L  I  E
100

        AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA

    241 ---+---------+---------+---------+---------+---------+---------+------
300

        TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAAACTAACTT

        A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K  T  Y  N
120

        GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC

    301 ---+---------+---------+---------+---------+---------+---------+------
360

        CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

        Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V  N  S  G
140

        TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGGTTAACTCTGGT

    361 ---+---------+---------+---------+---------+---------+---------+------
420

        ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA

        I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F  I  R  A
160

        ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT

    421 ---+---------+---------+---------+---------+---------+---------+------
480
```

```
        TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

        S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A
180

        TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT

    481 ---+---------+---------+---------+---------+---------+---------+------
540

        AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

        K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  N  V  I  I
200

        AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT

    541 ---+---------+---------+---------+---------+---------+---------+------
600

        TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAATTGCACTAGTAA

        P  E  G  S  G  Y  N  N  T  L  D  H  G  T  C  T  A  F  E  D
220

        CCAGAAGGATCCGGTTACAACAACACTTTGGACCACGGTACTTGTACTGCTTTCGAAGAC

    601 ---+---------+---------+---------+---------+---------+---------+------
660

        GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGACGAAAGCTTCTG

        S  E  L  G  D  D  V  E  A  N  F  T  A  L  F  A  P  A  I  R
240

        TCTGAATTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTCGCTCCAGCTATTAGA

    661 ---+---------+---------+---------+---------+---------+---------+------
720
```

```
        AGACTTAATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTCGATAATCT

            A  R  L  E  A  D  L  P  G  V  T  L  T  D  E  D  V  V  Y  L
260

        GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG
    721 ---+---------+---------+---------+---------+---------+---------+------
780

        CGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGCAACAAATGAAC

            M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  E  L  S  P
280

        ATGGACATGTGTCCATTCGACACTGTCGCTAGAACTTCTGACGCTACTGAATTGTCTCCA
    781 ---+---------+---------+---------+---------+---------+---------+------
840

        TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT

            F  C  A  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G
300

        TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT
    841 ---+---------+---------+---------+---------+---------+---------+------
900

        AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA

            K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  A
320

        AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT
    901 ---+---------+---------+---------+---------+---------+---------+------
960
```

```
          TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCGA

          N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  T  N  H
340

          AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC

     961 ---+---------+---------+---------+---------+---------+---------+------
1020

          TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

          T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  D  F  S
360

          ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT

    1021 ---+---------+---------+---------+---------+---------+---------+------
1080

          TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

          H  D  N  T  M  I  S  I  F  F  A  L  G  L  Y  N  G  T  K  P
380

          CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA

    1081 ---+---------+---------+---------+---------+---------+---------+------
1140

          GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTCGGT

          L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A  S  W  T
400

          TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT

    1141 ---+---------+---------+---------+---------+---------+---------+------
1200
```

```
        AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

        V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  Q  A  E  K  E  P
420

        GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA

    1201 ---+---------+---------+---------+---------+---------+---------+------
1260

        CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTCGACTTTTCCTTGGT

        L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A  V  D  K
440

        TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG

    1261 ---+---------+---------+---------+---------+---------+---------+------
1320

        AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACTGTTC

        L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G
460

        TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT

    1321 ---+---------+---------+---------+---------+---------+---------+------
1380

        AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

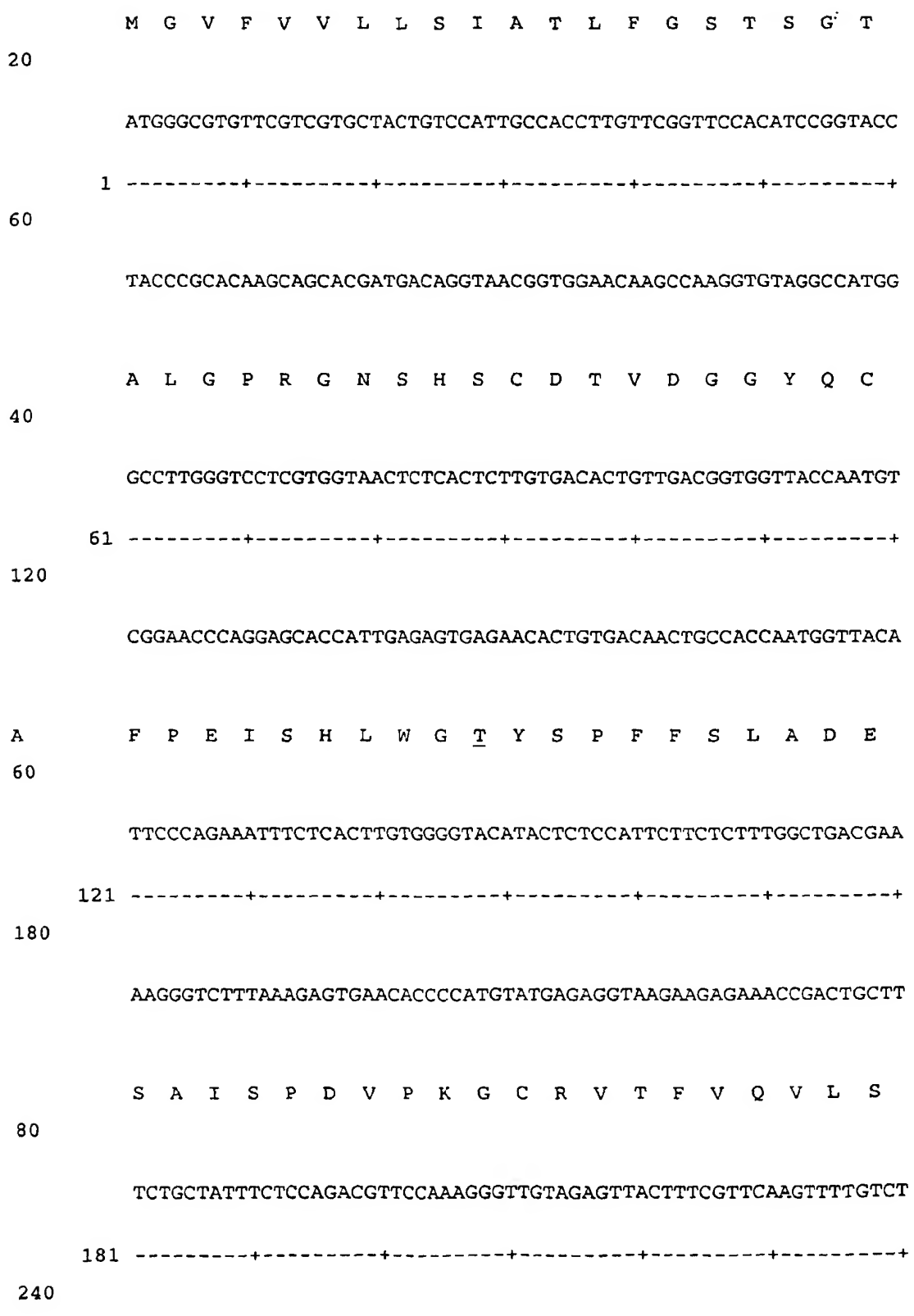
        N  W  A  E  C  F  A  *    467

        AACTGGGCTGAATGTTTCGCTTAA

    1381 ---+---------+---------+ 1410

        TTGACCCGACTTACAAAGCGAATT
```

Figure 9


M G V F V V L L S I A T L F G S T S G T
20
ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC
1 ---------+---------+---------+---------+---------+---------+
60
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG
A L G P R G N S H S C D T V D G G Y Q C
40
GCCTTGGGTCCTCGTGGTAACTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
61 ---------+---------+---------+---------+---------+---------+
120
CGGAACCCAGGAGCACCATTGAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA
A F P E I S H L W G T Y S P F F S L A D E
60
TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA
121 ---------+---------+---------+---------+---------+---------+
180
AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT
S A I S P D V P K G C R V T F V Q V L S
80
TCTGCTATTTCTCCAGACGTTCCAAAGGGTTGTAGAGTTACTTTCGTTCAAGTTTTGTCT
181 ---------+---------+---------+---------+---------+---------+
240

AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA

R H G A R Y P T S S A S K A Y S A L I E

100

AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCGTACTCTGCTTTGATTGAA

241 ---------+---------+---------+---------+---------+---------+

300

TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGCATGAGACGAAACTAACTT

A I Q K N A T A F K G K Y A F L K T Y N

120

GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC

301 ---------+---------+---------+---------+---------+---------+

360

CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

A Y T L G A D D L T P F G E Q Q M V N S G

140

TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAACAACAAATGGTTAACTCTGGT

361 ---------+---------+---------+---------+---------+---------+

420

ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACCAATTGAGACCA

I K F Y R R Y K A L A R K I V P F I R A

160

ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT

421 ---------+---------+---------+---------+---------+---------+

480

```
        TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

        S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A
180

        TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT

    481 ---------+---------+---------+---------+---------+---------+
540

        AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

        K  L  A  D  P  G  A  N  P  H  Q  A  S  P  V  I  N  V  I  I
200

        AAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTAACGTTATTATT

    541 ---------+---------+---------+---------+---------+---------+
600

        TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA

        P  E  G  A  G  Y  N  N  T  L  D  H  G  L  C  T  A  F  E  E
220

        CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA

    601 ---------+---------+---------+---------+---------+---------+
660

        GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT

        S  E  L  G  D  D  V  E  A  N  F  T  A  V  F  A  P  P  I  R
240

        TCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTCCACCAATTAGA

    661 ---------+---------+---------+---------+---------+---------+
720
```

```
        AGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT

        A  R  L  E  A  H  L  P  G  V  N  L  T  D  E  D  V  V  N  L
260

        GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTTAACTTG
    721 ---------+---------+---------+---------+---------+---------+
780

        CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC

        M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  Q  L  S  P
280

        ATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTCAATTGTCTCCA
    781 ---------+---------+---------+---------+---------+---------+
840

        TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT

        F  C  D  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G
300

        TTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT
    841 ---------+---------+---------+---------+---------+---------+
900

        AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA

        K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  V
320

        AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT
    901 ---------+---------+---------+---------+---------+---------+
960
```

TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCAA

N E L I A R L T H S P V Q D H T S T N H

340

AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC

961 ---------+---------+---------+---------+---------+---------+

1020

TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

T L D S N P A T F P L N A T L Y A D F S

360

ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT

1021 ---------+---------+---------+---------+---------+---------+

1080

TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

H D N T M V S I F F A L G L Y N G T K P

380

CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA

1081 ---------+---------+---------+---------+---------+---------+

1140

GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCGGT

L S T T S V E S I E E T D G Y S A S W T

400

TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT

1141 ---------+---------+---------+---------+---------+---------+

1200

```
        AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

        V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  E  A  E  K  E  P
420

        GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA

    1201 ---------+---------+---------+---------+---------+---------+
1260

        CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT

        L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  G  V  D  K
440

        TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGGTGTTGACAAG

    1261 ---------+---------+---------+---------+---------+---------+
1320

        AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACCACAACTGTTC

        L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G
460

        TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT

    1321 ---------+---------+---------+---------+---------+---------+
1380

        AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

        N  W  E  E  C  F  A  *    467

        AACTGGGAAGAATGTTTCGCTTAA

    1381 ---------+---------+---- 1404

        TTGACCCTTCTTACAAAGCGAATT
```

Figure 10

```
    M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T   20

    ATGGGGGTTTTCGTCGTTCTATTATCTATCGCGACTCTGTTCGGCAGCACATCGGGCACT

  1 ---------+---------+---------+---------+---------+---------+ 60

    TACCCCCAAAAGCAGCAAGATAATAGATAGCGCTGAGACAAGCCGTCGTGTAGCCCGTGA

    A  L  G  P  R  G  N  H  S  K  S  C  D  T  V  D  L  G  Y  Q   40

    GCGCTGGGCCCCCGTGGAAATCACTCCAAGTCCTGCGATACGGTAGACCTAGGGTACCAG

 61 ---------+---------+---------+---------+---------+---------+ 120

    CGCGACCCGGGGGCACCTTTAGTGAGGTTCAGGACGCTATGCCATCTGGATCCCATGGTC

    C  S  P  A  T  S  H  L  W  G  T  Y  S  P  Y  F  S  L  E  D   60

    TGCTCCCCTGCGACTTCTCATCTATGGGGCACGTACTCGCCATaCTTTTCGCTCGAGGAC

121 ---------+---------+---------+---------+---------+---------+ 180

    ACGAGGGGACGCTGAAGAGTAGATACCCCGtgCATGAGCGGTAtGAAAAGCGAGCTCCTG

    E  L  S  V  S  S  K  L  P  K  D  C  R  I  T  L  V  Q  V  L   80

    GAGCTGTCCGTGTCGAGTAAGCTTCCCAAGGATTGCCGGATCACCTTGGTACAGGTGCTA

181 ---------+---------+---------+---------+---------+---------+ 240

    CTCGACAGGCACAGCTCATTCGAAGGGTTCCTAACGGCCTAGTGGAACCATGTCCACGAT

    S  R  H  G  A  R  Y  P  T  S  S  K  S  K  K  Y  K  K  L  I   100

    TCGCGCCATGGAGCGCGGTACCCAACCAGCTCCAAGAGCAAAAAGTATAAGAAGCTTaTt

241 ---------+---------+---------+---------+---------+---------+ 300
```

```
    AGCGCGGTACCTCGCGCCATGGGTTGGTCGAGGTTCTCGTTTTTCATATTCTTCGAAtAa

    T  A  I  Q  A  N  A  T  D  F  K  G  K  Y  A  F  L  K  T  Y   120

    ACGGCGATCCAGGCCAATGCCACCGACTTCAAGGGCAAGTacGCCTTTTTGAAGACGTAC

301 ---------+---------+---------+---------+---------+---------+ 360

    TGCCGCTAGGTCCGGTTACGGTGGCTGAAGTTCCCGTTCAtgCGGAAAAACTTCTGCATG

    N  Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  L  V  N  S   140

    AACTATACTCTGGGTGCGGATGACCTCACTCCCTTTGGGGAGCAGCAGCTGGTGAACTCG

361 ---------+---------+---------+---------+---------+---------+ 420

    TTGATATGAGACCCACGCCTACTGGAGTGAGGGAAACCCCTCGTCGTCGACCACTTGAGC

    G  I  K  F  Y  Q  R  Y  K  A  L  A  R  S  V  V  P  F  I  R   160

    GGCATCAAGTTCTACCAGAGGTACAAGGCTCTGGCGCGCAGTGTGGTGCCGTTTATTCGC

421 ---------+---------+---------+---------+---------+---------+ 480

    CCGTAGTTCAAGATGGTCTCCATGTTCCGAGACCGCGCGTCACACCACGGCAAATAAGCG

    A  S  G  S  D  R  V  I  A  S  G  E  K  F  I  E  G  F  Q  Q   180

    GCCTCAGGCTCGGACCGGGTTATTGCTTCGGGAGAGAAGTTCATCGAGGGGTTCCAGCAG

481 ---------+---------+---------+---------+---------+---------+ 540

    CGGAGTCCGAGCCTGGCCCAATAACGAAGCCCTCTCTTCAAGTAGCTCCCCAAGGTCGTC

    A  K  L  A  D  P  G  A  T  N  R  A  A  P  A  I  S  V  I  I   200

    GCGAAGCTGGCTGATCCTGGCGCGACGAACCGCGCCGCTCCGGCGATTAGTGTGATTATT

541 ---------+---------+---------+---------+---------+---------+ 600
```

```
    CGCTTCGACCGACTAGGACCGCGCTGCTTGGCGCGGCGAGGCCGCTAATCACACTAATAA

    P  E  S  E  T  F  N  N  T  L  D  H  G  V  C  T  K  F  E  A   220

    CCGGAGAGCGAGACGTTCAACAATACGCTGGACCACGGTGTGTGCACGAAGTTTGAGGCG

601 ---------+---------+---------+---------+---------+---------+ 660

    GGCCTCTCGCTCTGCAAGTTGTTATGCGACCTGGTGCCACACACGTGCTTCAAACTCCGC

    S  Q  L  G  D  E  V  A  A  N  F  T  A  L  F  A  P  D  I  R   240

    AGTCAGCTGGGAGATGAGGTTGCGGCCAATTTCACTGCGCTCTTTGCACCCGACATCCGA

661 ---------+---------+---------+---------+---------+---------+ 720

    TCAGTCGACCCTCTACTCCAACGCCGGTTAAAGTGACGCGAGAAACGTGGGCTGTAGGCT

    A  R  L  E  K  H  L  P  G  V  T  L  T  D  E  D  V  V  S  L   260

    GCTCGCctCGAGAAGCATCTTCCTGGCGTGACGCTGACAGACGAGGACGTTGTCAGTCTA

721 ---------+---------+---------+---------+---------+---------+ 780

    CGAGCGgaGCTCTTCGTAGAAGGACCGCACTGCGACTGTCTGCTCCTGCAACAGTCAGAT

    M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  S  Q  L  S  P   280

    ATGGACATGTGTcCGTTTGATACGGTAGCGCGCACCAGCGACGCAAGTCAGCTGTCACCG

781 ---------+---------+---------+---------+---------+---------+ 840

    TACCTGTACACAgGCAAACTATGCCATCGCGCGTGGTCGCTGCGTTCAGTCGACAGTGGC

    F  C  Q  L  F  T  H  N  E  W  K  K  Y  D  Y  L  Q  S  L  G   300

    TTCTGTCAACTCTTCACTCACAATGAGTGGAAGAAGTACgACTACCTTCAGTCCTTGGGC

841 ---------+---------+---------+---------+---------+---------+ 900
```

```
        AAGACAGTTGAGAAGTGAGTGTTACTCACCTTCTTCATGcTGATGGAAGTCAGGAACCCG

        K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  I  G  F  T   320

        AAGTACTACGGCTACGGCGCAGGCAACCCTCTGGGACCGGCTCAGGGGATAGGGTTCACC

    901 ---------+---------+---------+---------+---------+---------+ 960

        TTCATGATGCCGATGCCGCGTCCGTTGGGAGACCCTGGCCGAGTCCCCTATCCCAAGTGG

        N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S  T  N  S
340

        AACGAGCTGATTGCCCGGTTGACgCGTTCGCCAGTGCAGGACCACACCAGCACTAACTCG

    961 ---------+---------+---------+---------+---------+---------+
1020

        TTGCTCGACTAACGGGCCAACTGcGCAAGCGGTCACGTCCTGGTGTGGTCGTGATTGAGC

        T  L  V  S  N  P  A  T  F  P  L  N  A  T  M  Y  V  D  F  S
360

        ACTCTAGTCTCCAACCCGGCCACCTTCCCGTTGAACGCTACCATGTACGTCGACTTTTCA

   1021 ---------+---------+---------+---------+---------+---------+
1080

        TGAGATCAGAGGTTGGGCCGGTGGAAGGGCAACTTGCGATGGTACATGCAGCTGAAAAGT

        H  D  N  S  M  V  S  I  F  F  A  L  G  L  Y  N  G  T  E  P
380

        CACGACAACAGCATGGTTTCCATCTTCTTTGCATTGGGCCTGTACAACGGCACTGAACCC

   1081 ---------+---------+---------+---------+---------+---------+
1140

        GTGCTGTTGTCGTACCAAAGGTAGAAGAAACGTAACCCGGACATGTTGCCGTGACTTGGG
```

```
        L  S  R  T  S  V  E  S  A  K  E  L  D  G  Y  S  A  S  W  V
400

        TTGTCCCGGACCTCGGTGGAAAGCGCCAAGGAATTGGATGGGTATTCTGCATCCTGGGTG

    1141 ---------+---------+---------+---------+---------+---------+
1200

        AACAGGGCCTGGAGCCACCTTTCGCGGTTCCTTAACCTACCCATAAGACGTAGGACCCAC

        V  P  F  G  A  R  A  Y  F  E  T  M  Q  C  K  S  E  K  E  P
420

        GTGCCTTTCGGCGCGCGAGCCTACTTCGAGACGATGCAATGCAAGTCGGAAAAGGAGCCT

    1201 ---------+---------+---------+---------+---------+---------+
1260

        CACGGAAAGCCGCGCGCTCGGATGAAGCTCTGCTACGTTACGTTCAGCCTTTTCCTCGGA

        L  V  R  A  L  I  N  D  R  V  V  P  L  H  G  C  D  V  D  K
440

        CTTGTTCGCGCTTTGATTAATGACCGGGTTGTGCCACTGCATGGCTGCGATGTGGACAAG

    1261 ---------+---------+---------+---------+---------+---------+
1320

        GAACAAGCGCGAAACTAATTACTGGCCCAACACGGTGACGTACCGACGCTACACCTGTTC

        L  G  R  C  K  L  N  D  F  V  K  G  L  S  W  A  R  S  G  G
460

        CTGGGGCGATGCAAGCTGAATGACTTTGTCAAGGGATTGAGTTGGGCCAGATCTGGGGGC

    1321 ---------+---------+---------+---------+---------+---------+
1380

        GACCCCGCTACGTTCGACTTACTGAAACAGTTCCCTAACTCAACCCGGTCTAGACCCCCG
```

```
      N  W  G  E  C  F  S  *     467

      AACTGGGGAGAGTGCTTTAGTTGA

1381 ---------+---------+---- 1404

      TTGACCCCTCTCACGAAATCAACT
```

Figure 11

```
      CP-1

         Eco RI  M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T

      TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA

   1 ---------+---------+---------+---------+---------+---------+ 60

      ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

        S  G  T  A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G

      CATCCGGTACCGCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG

  61 ---------+---------+---------+---------+---------+---------+ 120

      GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC

          CP-2
               CP-3

        Y  Q  C  F  P  E  I  S  H  L  W  G  Q  Y  S  P  Y  F  S  L

      GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT

 121 ---------+---------+---------+---------+---------+---------+ 180

      CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA

        E  D  E  S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q

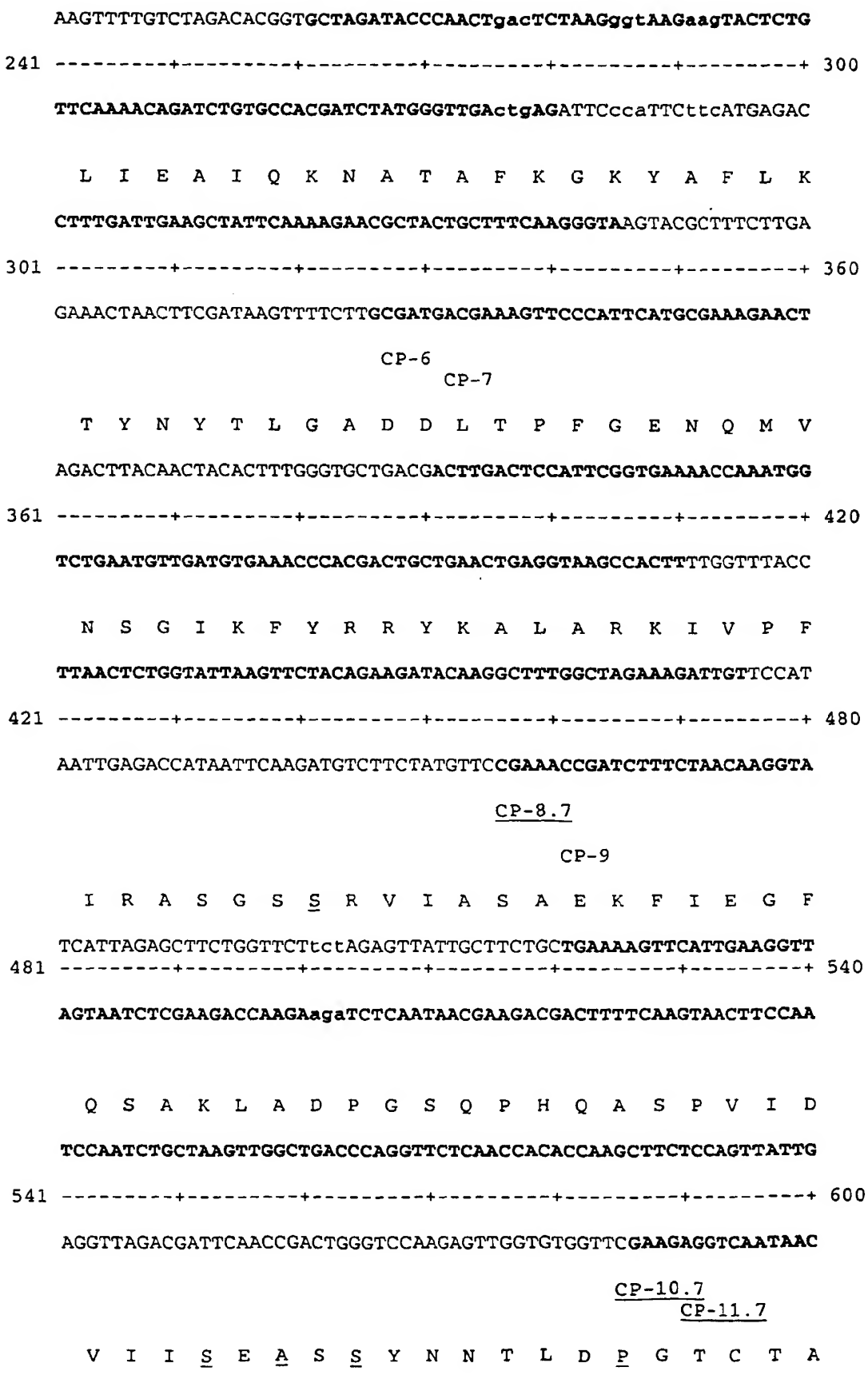
      TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTC

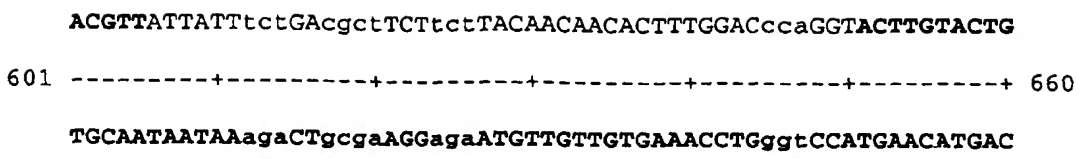
 181 ---------+---------+---------+---------+---------+---------+ 240

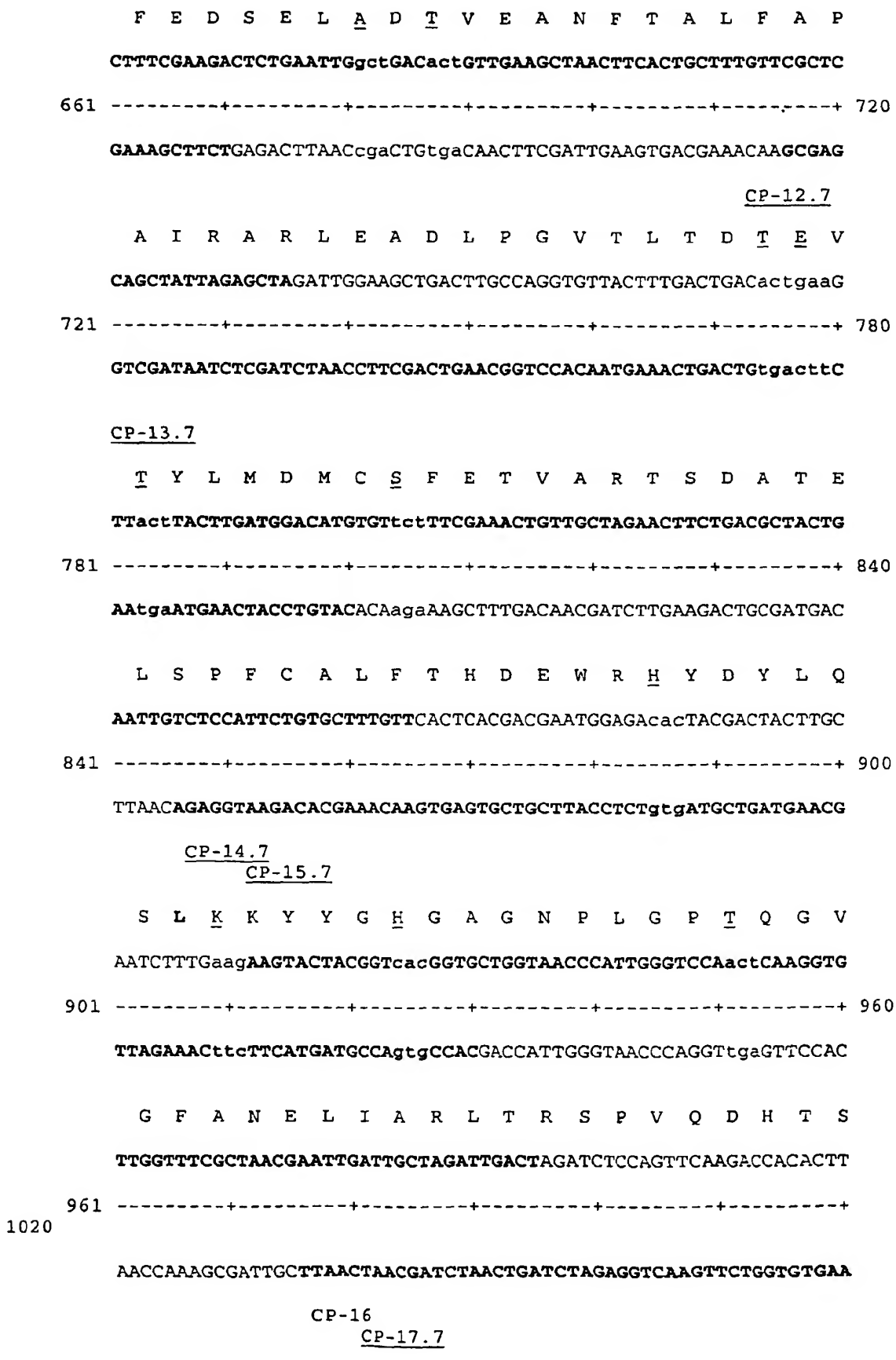
      ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG

                        CP-4.7
                             CP-5.7

        V  L  S  R  H  G  A  R  Y  P  T  D  S  K  G  K  K  Y  S  A
```


AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTgacTCTAAGggtAAGaagTACTCTG
241 ---------+---------+---------+---------+---------+---------+ 300
TTCAAAACAGATCTGTGCCACGATCTATGGGTTGActgAGATTCccaTTCttcATGAGAC
L I E A I Q K N A T A F K G K Y A F L K
CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
301 ---------+---------+---------+---------+---------+---------+ 360
GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT
CP-6
CP-7
T Y N Y T L G A D D L T P F G E N Q M V
AGACTTACAACTACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGG
361 ---------+---------+---------+---------+---------+---------+ 420
TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC
N S G I K F Y R R Y K A L A R K I V P F
TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
421 ---------+---------+---------+---------+---------+---------+ 480
AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
CP-8.7
CP-9
I R A S G S S R V I A S A E K F I E G F
TCATTAGAGCTTCTGGTTCTtctAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT
481 ---------+---------+---------+---------+---------+---------+ 540
AGTAATCTCGAAGACCAAGAagaTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA
Q S A K L A D P G S Q P H Q A S P V I D
TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
541 ---------+---------+---------+---------+---------+---------+ 600
AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAAC
CP-10.7
CP-11.7
V I I S E A S S Y N N T L D P G T C T A


ACGTTATTATTtctGAcgctTCTtctTACAACAACACTTTGGACccaGGTACTTGTACTG
601 ---------+---------+---------+---------+---------+---------+ 660
TGCAATAATAAagaCTgcgaAGGagaATGTTGTTGTGAAACCTGggtCCATGAACATGAC


F E D S E L A D T V E A N F T A L F A P
CTTTCGAAGACTCTGAATTGgctGACactGTTGAAGCTAACTTCACTGCTTTGTTCGCTC
661 ---------+---------+---------+---------+---------+---------+ 720
GAAAGCTTCTGAGACTTAACcgaCTGtgaCAACTTCGATTGAAGTGACGAAACAAGCGAG
CP-12.7
A I R A R L E A D L P G V T L T D T E V
CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACactgaaG
721 ---------+---------+---------+---------+---------+---------+ 780
GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGtgacttC
CP-13.7
T Y L M D M C S F E T V A R T S D A T E
TTacttACTTGATGGACATGTGTtcttTCGAAACTGTTGCTAGAACTTCTGACGCTACTG
781 ---------+---------+---------+---------+---------+---------+ 840
AAtgaATGAACTACCTGTACACAagaAAGCTTTGACAACGATCTTGAAGACTGCGATGAC
L S P F C A L F T H D E W R H Y D Y L Q
AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGAcacTACGACTACTTGC
841 ---------+---------+---------+---------+---------+---------+ 900
TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTgtgATGCTGATGAACG
CP-14.7
CP-15.7
S L K K Y Y G H G A G N P L G P T Q G V
AATCTTTGaagAAGTACTACGGTcacGGTGCTGGTAACCCATTGGGTCCAactCAAGGTG
901 ---------+---------+---------+---------+---------+---------+ 960
TTAGAAACttcTTCATGATGCCAgtgCCACGACCATTGGGTAACCCAGGTtgaGTTCCAC
G F A N E L I A R L T R S P V Q D H T S
TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT
961 ---------+---------+---------+---------+---------+---------+
1020
AACCAAAGCGATTGCTTAACTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA
CP-16
CP-17.7

```
          T  N  H  T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A

        CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG

    1021 ---------+---------+---------+---------+---------+---------+
1080

        GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

          D  F  S  H  D  N  G  I  I  S  I  F  F  A  L  G  L  Y  N  G

        CTGACTTCTCTCACGACAACggtattATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG

    1081 ---------+---------+---------+---------+---------+---------+
1140

        GACTGAAGAGAGTGCTGTTGccataaTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC

                                   CP-18.7
                                        CP-19.7

          T  A  P  L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  S

        GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTt

    1141 ---------+---------+---------+---------+---------+---------+
1200

        CATGACGAGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGAa

          A  W  T  V  P  F  A  S  R  A  Y  V  E  M  M  Q  C  Q  A  E

        ctgctTGGACTGTTCCATTCgcttctAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
    1201 ---------+---------+---------+---------+---------+---------+
1260

        gacgaACCTGACAAGGTAAGcgaagaTCTCGAATGCAACTTTACTACGTTACAGTTCGAC

                                              CP-20
                                                   CP-21

          K  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A

        AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG

    1261 ---------+---------+---------+---------+---------+---------+
1320

        TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC
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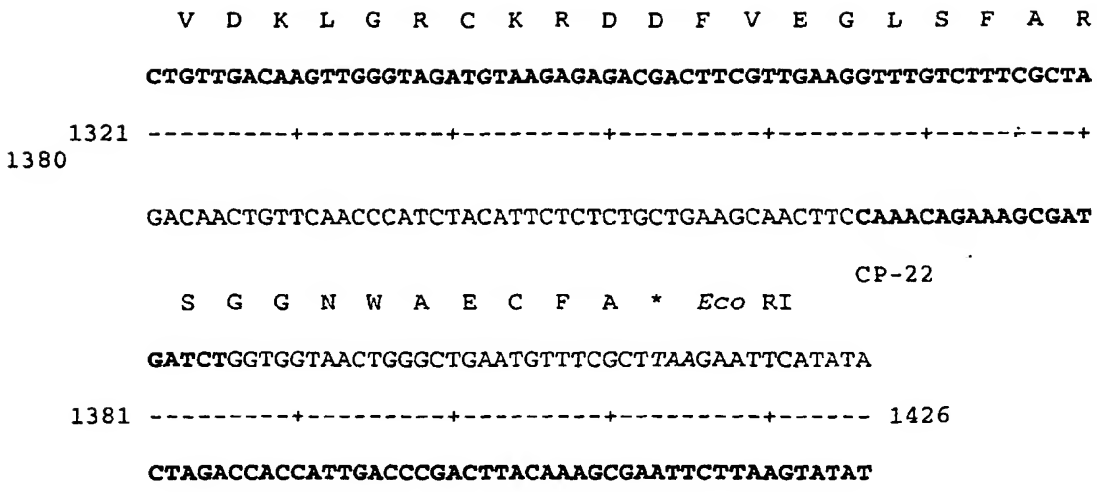

V D K L G R C K R D D F V E G L S F A R
CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA
1321 ---------+---------+---------+---------+---------+---------+
1380
GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
CP-22
S G G N W A E C F A * Eco RI
GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
1381 ---------+---------+---------+---------+------ 1426
CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT

Figure 12

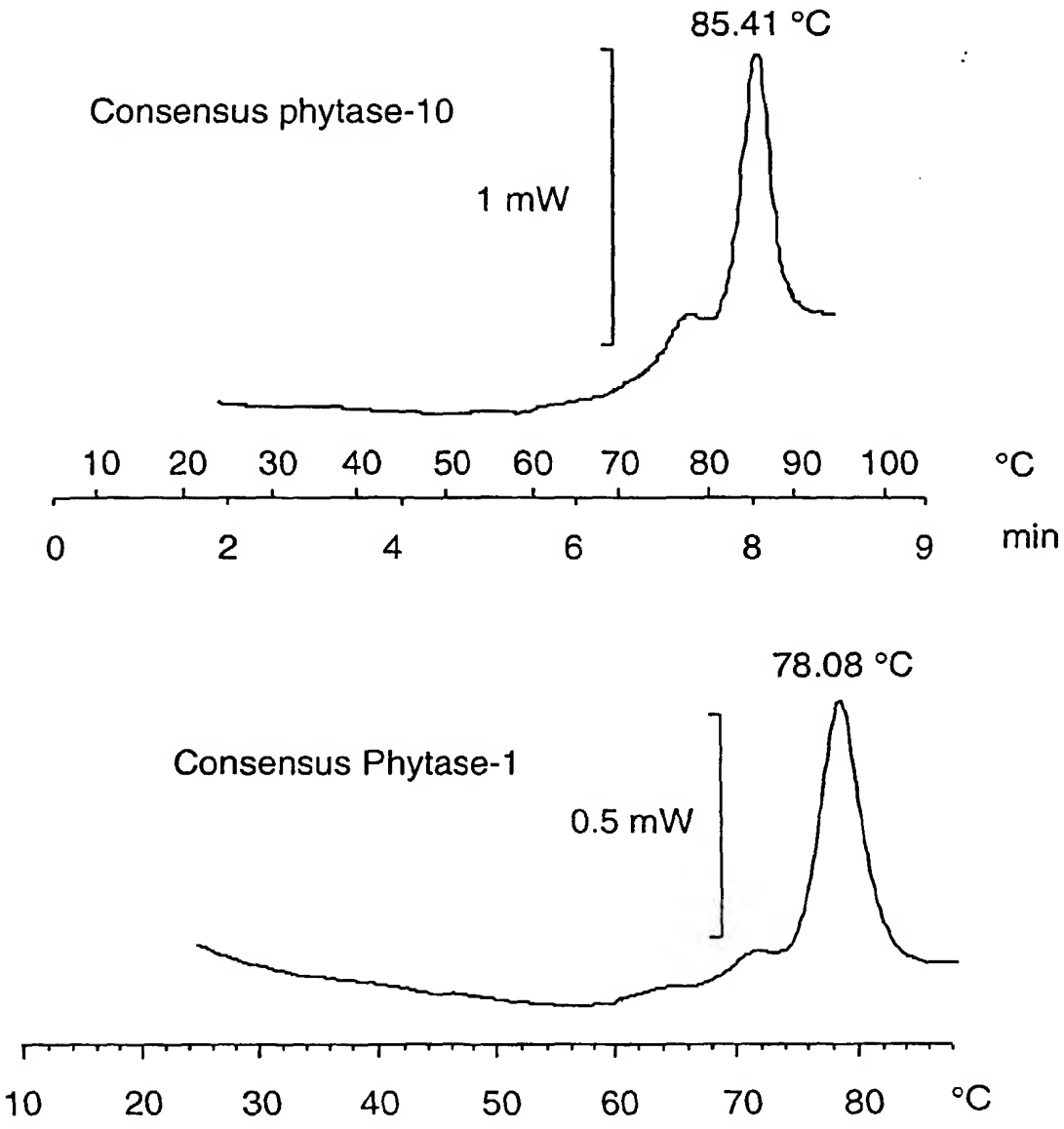

85.41 °C
Consensus phytase-10
1 mW
10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min
78.08 °C
Consensus Phytase-1
0.5 mW
10 20 30 40 50 60 70 80 °C

Figure 13

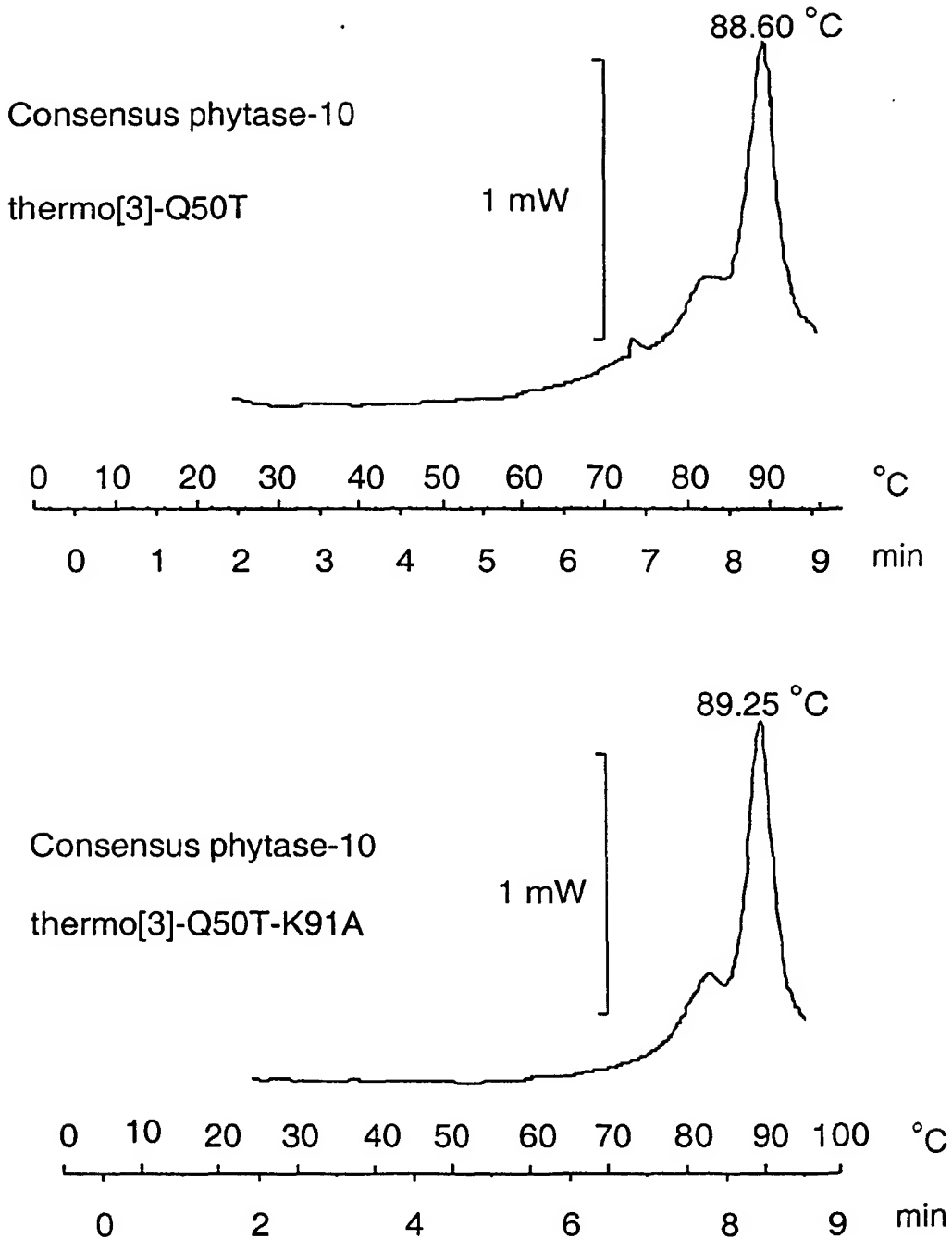

88.60 °C
Consensus phytase-10
thermo[3]-Q50T
1 mW
0 10 20 30 40 50 60 70 80 90 °C
0 1 2 3 4 5 6 7 8 9 min
89.25 °C
Consensus phytase-10
thermo[3]-Q50T-K91A
1 mW
0 10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min

Figure 14

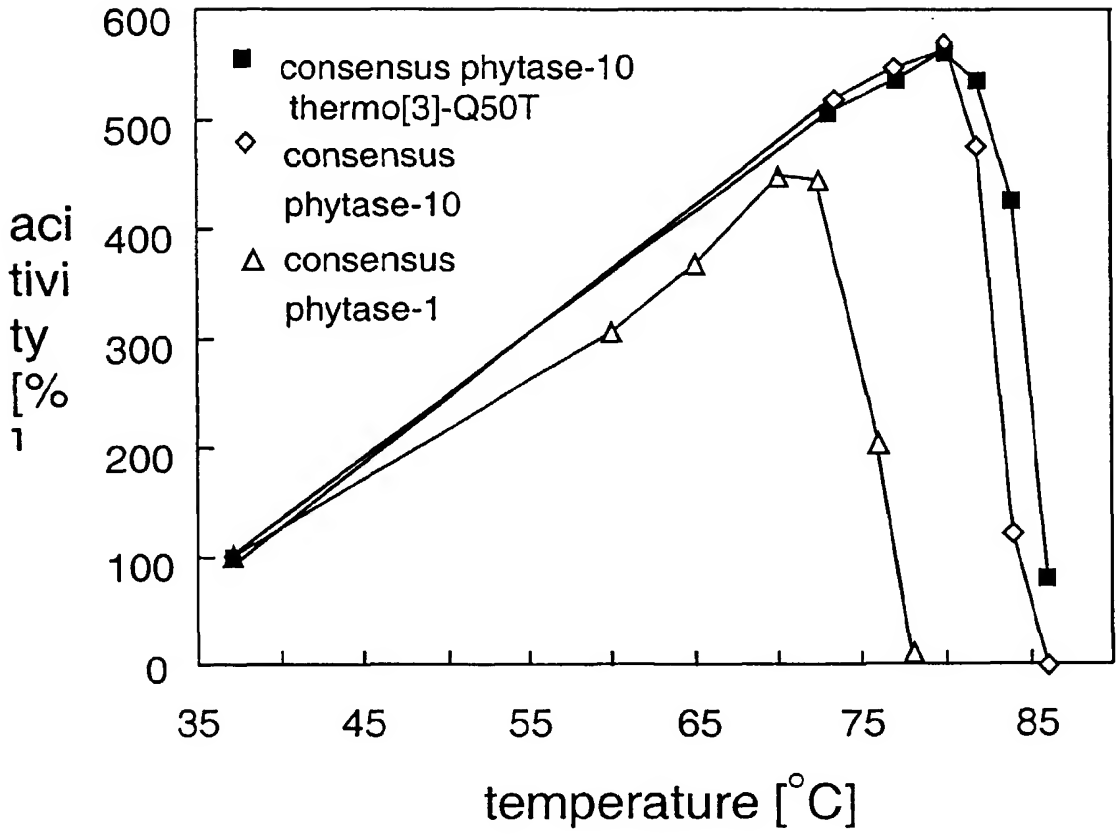

600
500
400
300
200
100
0
aci
tivi
ty
[%
1
consensus phytase-10 thermo[3]-Q50T
consensus phytase-10
consensus phytase-1
35
45
55
65
75
85
temperature [°C]

Figure 15

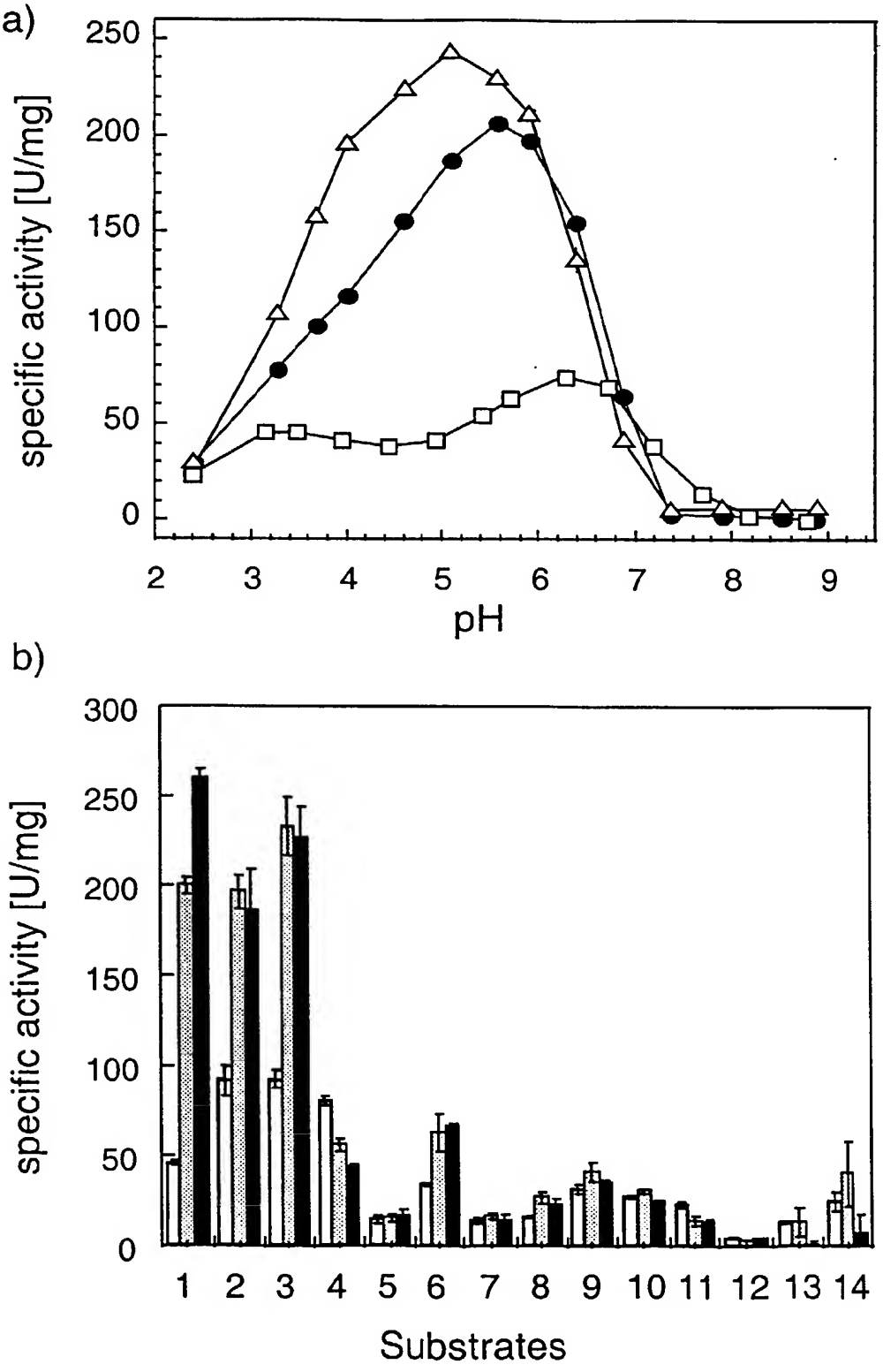
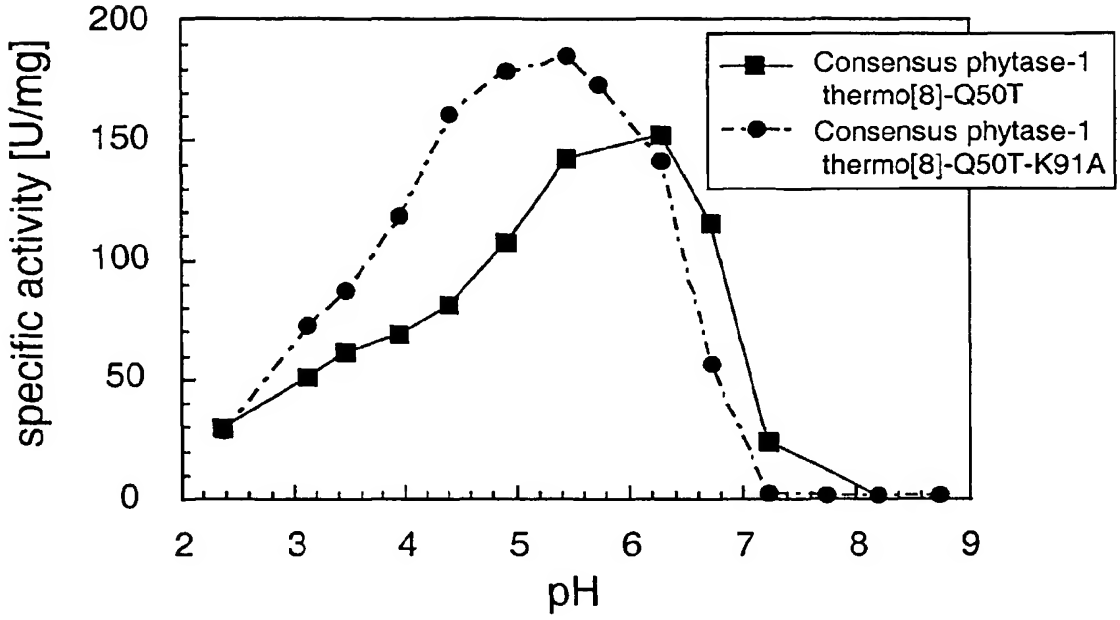

a)
specific activity [U/mg]
250
200
150
100
50
0
2
3
4
5
6
7
8
9
pH
b)
specific activity [U/mg]
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Substrates

Figure 16


specific activity [U/mg]
200
150
100
50
0
2
3
4
5
6
7
8
9
pH
Consensus phytase-1 thermo[8]-Q50T
Consensus phytase-1 thermo[8]-Q50T-K91A

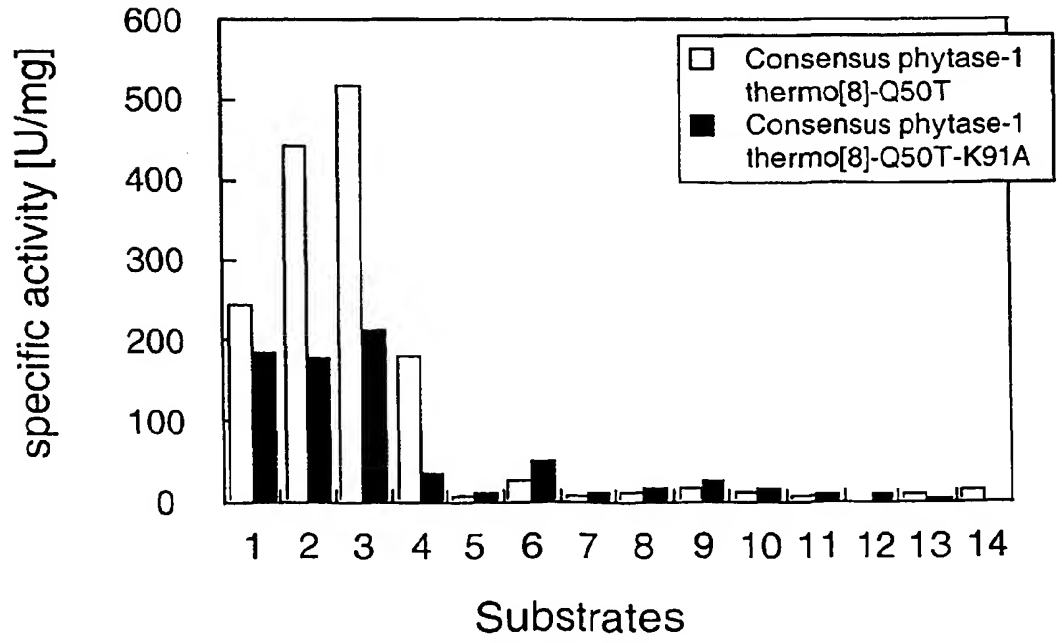

specific activity [U/mg]
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Substrates
Consensus phytase-1 thermo[8]-Q50T
Consensus phytase-1 thermo[8]-Q50T-K91A

Figure 17

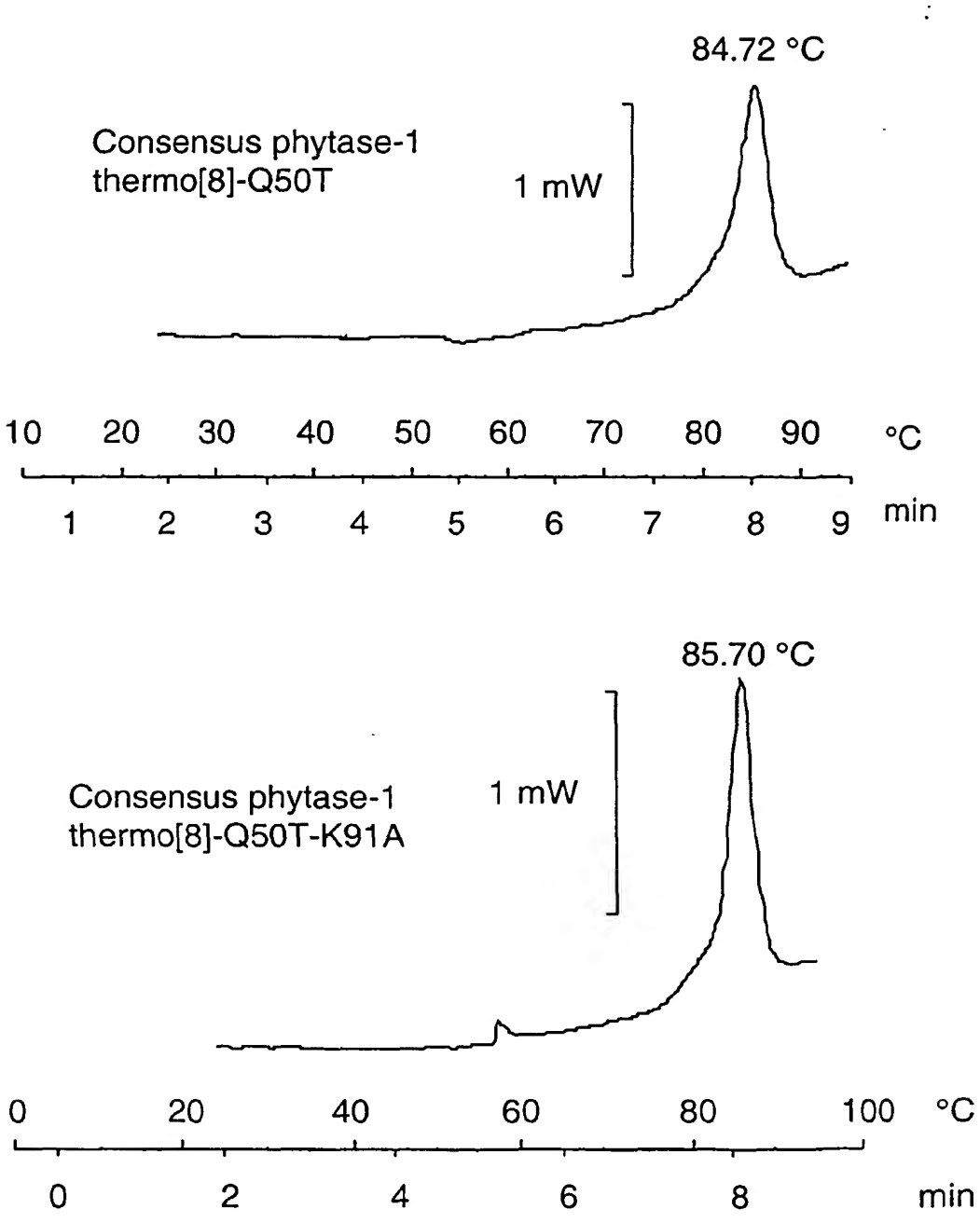

84.72 °C
Consensus phytase-1
thermo[8]-Q50T
1 mW
10 20 30 40 50 60 70 80 90 °C
1 2 3 4 5 6 7 8 9 min
85.70 °C
Consensus phytase-1
thermo[8]-Q50T-K91A
1 mW
0 20 40 60 80 100 °C
0 2 4 6 8 min

Figure 18

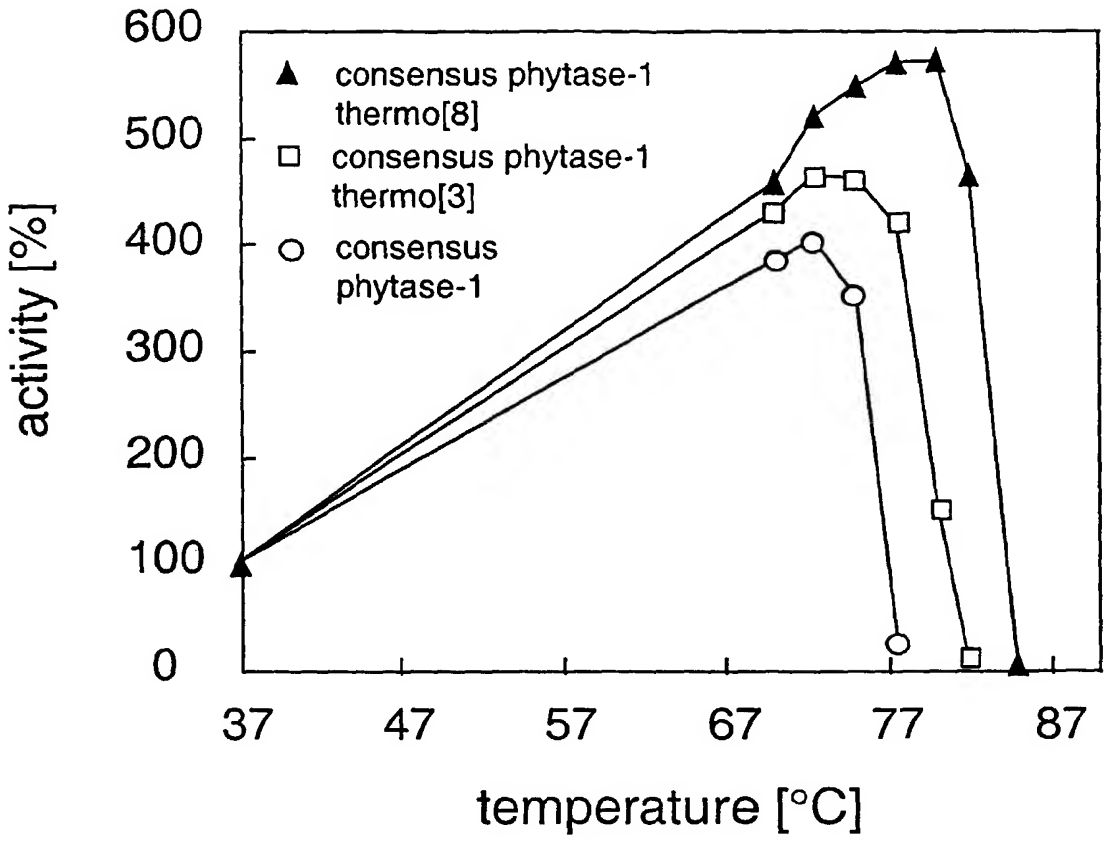
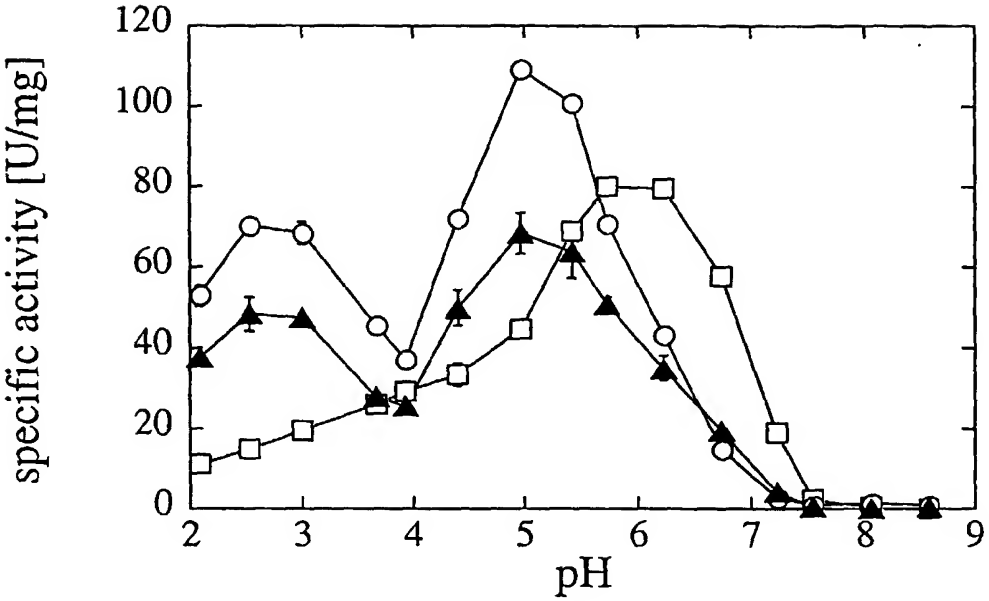

600
500
400
300
200
100
0
activity [%]
consensus phytase-1 thermo[8]
consensus phytase-1 thermo[3]
consensus phytase-1
37
47
57
67
77
87
temperature [°C]

Figure 19


specific activity [U/mg]
120
100
80
60
40
20
0
2
3
4
5
6
7
8
9
pH

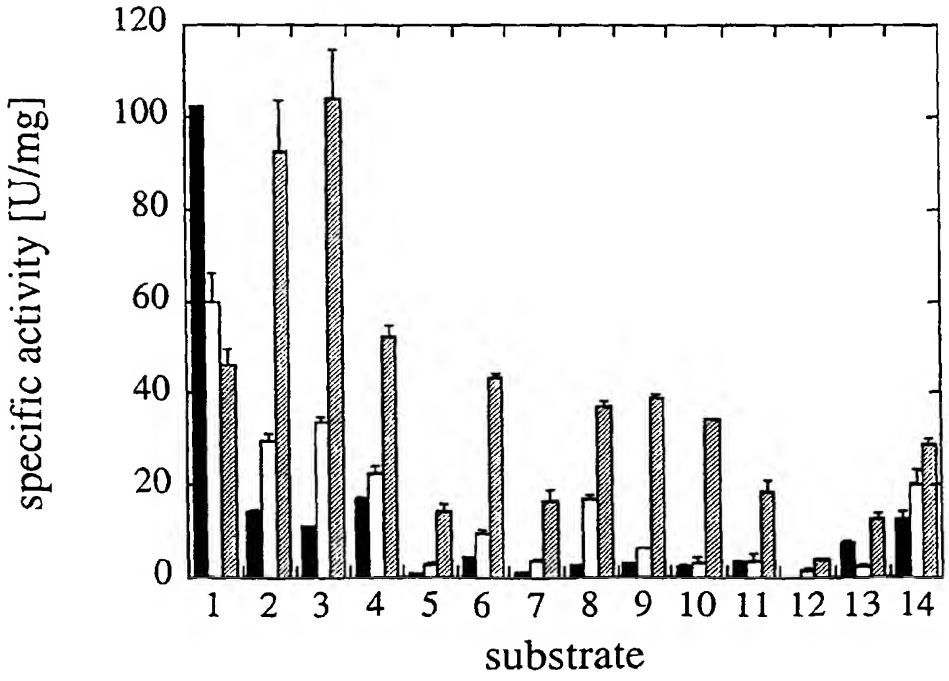

specific activity [U/mg]
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
substrate

Figure 20

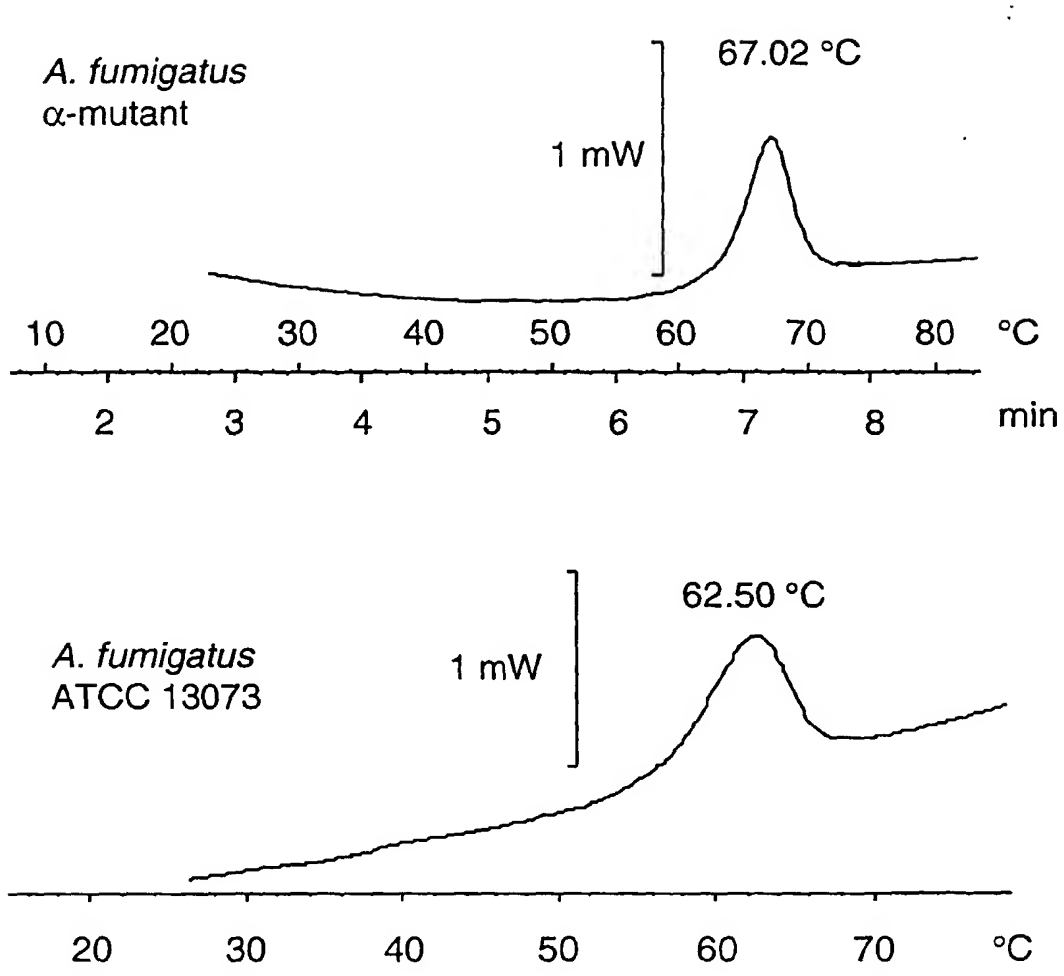

A. fumigatus
α-mutant
67.02 °C
1 mW
10
20
30
40
50
60
70
80
°C
2
3
4
5
6
7
8
min
A. fumigatus
ATCC 13073
62.50 °C
1 mW
20
30
40
50
60
70
°C

Figure 21

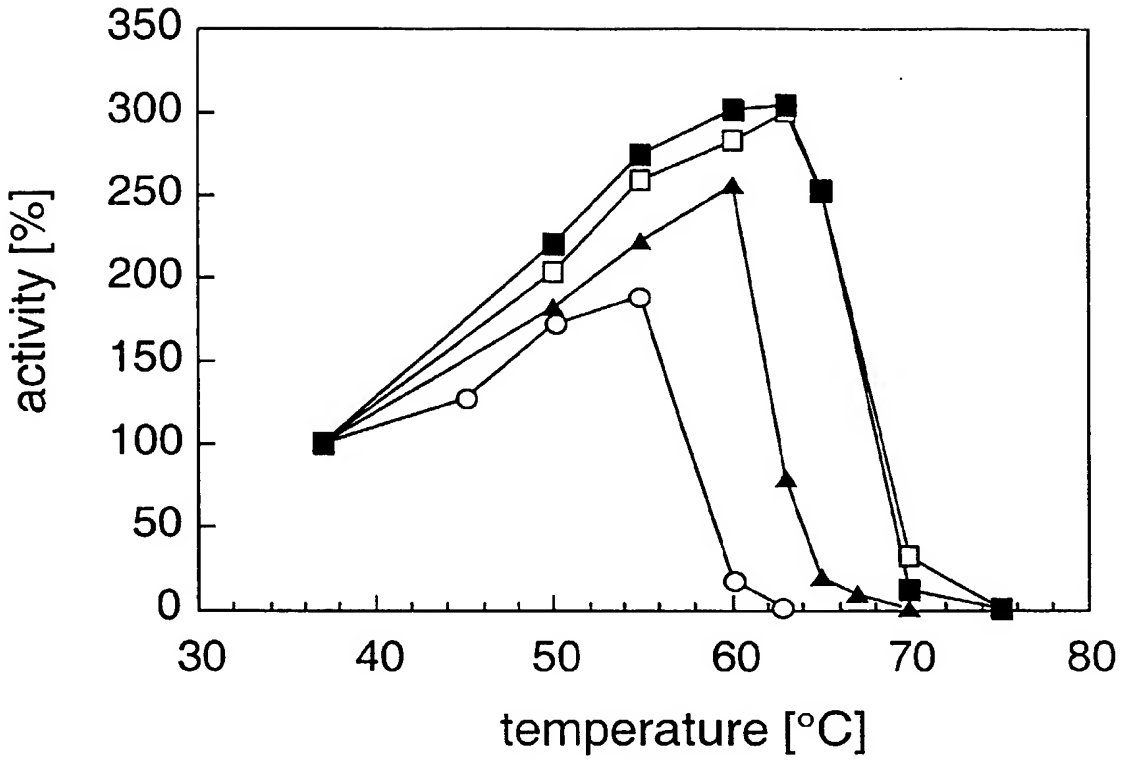

350
300
250
200
150
100
50
0
activity [%]
30
40
50
60
70
80
temperature [°C]

Figure 22

1 MGVFVVLLSI ATLFGSTSGT ALGPRGNSHS CDTVDGGYQC FPEISSNWSP

51 YSPYFSLADE SAISPDVPKG CRVTFVQVLQ RHGARFPTSG AATRISALIE

101 AIQKNATAFK GKYAFLKTYN YTLGADDLVP FGANQSSQAG IKFYRRYKAL

151 ARKIVPFIRA SGSDRVIDSA TNWIEGFQSA KLADPGANPH QASPVINVII

201 PEGAGYNNTL DHGLCTAFEE SELGDDVEAN FTAVFAPPIR ARLEAHLPGV

251 NLTDEDVVNL MDMCPFDTVA RTSDATELSP FCDLFTHDEW IQYDYLGDLD

301 KYYGTGAGNP LGPAQGVGFV NELIARLTHS PVQDHTSTNH TLDSNPATFP

351 LNATLYADFS HDNTMVAIFF ALGLYNGTKP LSTTSVESIE ETDGYSASWL

401 VPFSARMYVE MMQCEAEKEP LVRVLVNDRV VPLHGCGVDK LGRCKRDDFV

451 EGLSFARSGG NWEECFA